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THE AMERICAN JOURNAL OF Clinical Nutrition

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The Effects of Nutritional Deficiency on the Heart: A Review

By RICHARD H. FOLLIS, JR., M.D.

THE INTEGRITY of the myocardium, both functional and anatomical, is dependent on its nutrition. Obviously, the heart differs in no way from other tissues.[1] Therefore, single or multiple deficiencies of the various essential nutrients: elements, amino acids, vitamins, or fatty acids, may be expected to have profound consequences on the myocardium. The effects of lack of oxygen supply are too obvious to require further comment. It is unlikely that a total lack of substrate, i.e., glucose, pyruvate, lactate, acetate, etc., ever occurs. However, in several naturally occurring disease states in man, as well as artificially produced situations in experimental animals, the importance of certain essential nutrients for the normal function and structure of the heart may be demonstrated.

In the following discussion we should like first to take up changes which may be produced in the experimental animal and then to indicate how such findings may be applied to an understanding of the changes in the human. For convenience, we shall discuss deficiencies of inorganic elements, amino acids (and protein), vitamins, and fatty acids, in that order. Furthermore, it should be obvious that there are a number of instances in which physiological alterations may be observed in the absence of anatomical changes in the tissues. These will also be cited.

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INORGANIC ELEMENTS

Potassium

In any discussion of the effects of the inorganic environment on the integrity of the heart muscle fiber one naturally turns to the studies of Sidney Ringer in the 1880's.[3] Even before the development of our knowledge of ionization by Arrhenius, Ringer demonstrated the importance of the fine balance between sodium, potassium, and calcium for the *in vitro* function of the frog heart. Although certain physiological alterations, with particular respect to the electrocardiogram, have been reported for all these elements, most attention has been given to the effects of potassium deficiency on the myocardium. This is because profound alterations may be produced when various species are placed on diets deficient in this electrolyte. Thus far, changes have been described in rats, mice, dogs, swine, and calves; the first species has been most extensively studied.

In the growing rat certain critical levels of potassium in the diet must be reached before morphologic alterations appear. This has been set at about 0.17 per cent.[4] As will be noted below, the sodium content is of importance in this respect, too. In the young, actively-growing rat morphologic changes appear as early as the eighth day.[5] The first alteration is a loss of striation of the fibers, which assume a hyaline appearance. Following this, the fibers disintegrate. The sarcolemma nuclei become pyknotic; leukocytes infiltrate

the area. Single fibers or groups are usually involved, though one may encounter large areas comprising an entire low-power field. All such areas, large or small, are then infiltrated by connective tissue cells. Fine reticulum fibers appear, and these in time take on the more mature qualities of hyaline collagen. It is of interest that certain muscle fibers seem to withstand the deleterious effects of low potassium concentration longer than others. In certain rats carried on a low potassium regimen for as long as 327 days fresh lesions have been found side by side with old healed scars.

It will doubtless be recalled that Ringer[6] studied the effects of rubidium and cesium on the frog heart, showing that rubidium more resembled potassium, while cesium acted more like barium. When rubidium is substituted for potassium in a low potassium ration no myocardial necroses appear, while cesium fails to protect.[7] Neither group of animals lives very long since they succumb in peculiar fits.

It would appear unnecessary to detail the effects of potassium deficiency in other species. However, some recent and interesting observations of Cannon and associates[8] should be mentioned. These investigators found that the effects of potassium deficiency on the myocardium can be profoundly affected by sodium chloride, either in the diet or when administered by subcutaneous injection. In the latter case particularly, death occurs suddenly. On microscopic examination extensive necrosis and disintegration of the muscle fibers are found.[9] A prominent feature is the absence of cellular reaction. The lesions would appear to be far more extensive than those which we[5] and others have reported in rats. Cannon *et al.*,[8] therefore, raise the question as to whether such changes may be due not to potassium deficiency but to sodium toxicity. This is an intriguing concept and warrants further study. These interrelations of sodium and potassium in various situations have been discussed by Cannon and co-workers.[8]

It should be pointed out that no studies have ever been reported in which concentrations of sodium and potassium in the tissues and serum have been correlated with each other and with physiologic alterations (as seen on electrocardiograms) and morphologic changes in the myocardium. Such data should be most interesting, particularly in pointing the way to studies in human disease.

In man potassium depletion is usually conditioned by some disturbance in the absorption or excretion of this ion. However, deliberate attempts have been made to produce experimental potassium deficiency in the human by dietary restriction. Thus, Black and Milne[10] placed two subjects on a low potassium regimen. The serum concentrations of the ion fell and the serum bicarbonate rose. Sodium was retained; there was an expansion of extracellular fluid and an equivalent shrinkage of intracellular fluid. No very striking clinical symptoms appeared. The lack of these may have been due to the fact that there were no other primary metabolic defects which might interfere with "normal" adaptive mechanisms.

In contrast, the profound effects which accompany the hypokalemia of certain clinical syndromes are well known and becoming better understood. Such states of potassium depletion represent conditioned deficiencies and may be brought about by severe diarrhea,[11] during the treatment of diabetes mellitus,[12] in Cushing's syndrome,[13] during therapy with desoxycorticosterone,[14] and in certain types of chronic nephritis.[15] Changes in the electrocardiogram indicate disturbances in the physiologic activity of the myocardium.[16] Morphologic alterations in the heart have been described only very occasionally, and whether they represent changes specifically produced by potassium deficiency alone or in combination with some other metabolic defect cannot be stated with any certainty.

Magnesium

Although the addition of magnesium to solutions containing sodium, potassium, and calcium appears to have some effect on contraction of the smooth muscle of the intestine, the physiologic activity of heart does not appear to be affected, at least *in vitro*, by changing the concentration of this element.[17] The classic studies of McCollum and his group [18,19] on diets deficient in magnesium clearly showed the indispensability of this element for rats and

dogs. The possible relation of magnesium to the heart was indicated by changes in the rate in rats and in vascular abnormalities following the characteristic convulsive seizures of magnesium deficiency. Sino-auricular block has been seen on electrocardiographic examination.[20] No histologic alterations have been described in the myocardium, however, until recently. An important study of magnesium deficiency has been reported in calves by Blaxter *et al.*[21] On a ration low in this element the animals become hyperirritable, develop increased pulse rates, and finally die in convulsions. Most interesting are the alterations in the heart. On gross inspection one sees hemorrhagic areas, which, under the microscope, appear to be related to thrombi in the small vessels of the myocardium; foci of necrosis are found in the muscle. Further studies, particularly with relation to more details as to the pathogenesis of the changes, will be awaited with interest. It would appear, therefore, that the physiologic alterations already described may now be correlated with definite morphologic changes. A restudy of the heart of other species suffering from magnesium deficiency is clearly indicated.

Although occasional instances of magnesium tetany in man have been reported, no studies relative to the heart have been presented.

Calcium

One of Ringer's[3] fundamental contributions was to show that when calcium salts were added to a solution of sodium chloride in which the isolated frog's heart had ceased to beat, the excitability to stimuli returned and was followed by spontaneous beats. Relaxation, however, was delayed and imperfect, so that a tonic or systolic state ensued.

It, is therefore, not surprising that physiologic alterations might be found *in vivo* when animals are placed on calcium-deficient diets, so that serum calcium values fall to low levels. No histologic changes have been described, however, in the myocardium of experimental animals on calcium-deficient regimens.[22]

In man a reduction in serum calcium may occur under a variety of conditions: poor absorption, excessive renal excretion, disturbances in parathyroid function—all may lead to diminished extracellular concentrations of calcium. Such states of hypocalcemia, particularly when serum levels reach 7 mg per 100 ml or below, are accompanied by changes in the electrocardiogram.[23] No anatomic alterations in the myocardium have been described.

Sodium

The effects of sodium on the contractility of the frog's heart were studied by Ringer.[3] Experimental diets, deficient in sodium, have failed to produce morphologic changes in the heart muscle of rats.[24]

Copper

Copper deficiency has been shown to lead to a decrease in cytochrome oxidase activity of heart muscle.[25] In laboratory experience, as far as we are aware, no morphologic changes have been observed in animals placed on a low copper regimen.

However, a syndrome, "falling disease," has been described in Australia among cattle pastured in areas whose soil is deficient in copper.[26] The animals develop changes in the electrocardiogram and may die suddenly. Microscopic examination of the myocardium reveals "atrophy with replacement fibrosis." The pathogenesis has not been studied at all in detail.

Iron

The general effects of anemia on the heart are well recognized.[27] So, too, the relation of iron to hemoglobin formation and red blood cell production is clear enough. Despite the profound effects of iron deficiency on the erythrocyte, there is little or no evidence that a reduction in concentration of the mineral in the intercellular fluid adversely affects the integrity of heart muscle fibers. Because of the presence of iron in cardiac myohemoglobin one might have expected that alterations might be found. It would appear that muscle fibers have the first call on iron, in contrast to the developing erythron. Whipple and his associates[28] have shown this to be the case. In our own experience[29] and that of others no myocardial alterations have been observed in severely iron-deficient animals. The patho-

TABLE I

Effects of Deficiency of Elements on the Heart

Element	Functional change	Anatomical change
Potassium	+(3)*	+(5, 8, 9)*
Magnesium	+(18, 19, 20)	+(21)
Calcium	+(23)	0 (22)
Sodium	+(3)	0 (24)
Copper	+(25)	+(26)
Iron	—	—
Iodine	T†	T†
Cobalt	—	? (30)
Zinc	—	0 (33)
Phosphorus	—	0 (32)
Manganese	—	0 (34)
Chlorine	—	0 (31)

* Numbers in parentheses refer to reports listed in References.

† Theoretical.

logic findings of "fatty infiltration" and "degeneration" which have been noted in human material associated with anemia can only be mentioned. The exact pathogenesis is completely obscure.

Cobalt

Cobalt is, of course, related to blood formation inasmuch as it is a part of the vitamin B_{12} molecule. No metabolic alterations as a result of cobalt deficiency have as yet been described in the heart. More pointed investigations must be carried out, because calves suffering from "enzootic marasmus" in Florida where the cobalt content of the soil is low may exhibit "degenerative" changes in the heart.[30]

Miscellaneous Elements

Besides the elements referred to specifically above, there are others which are indispensable for the mammalian organism. As yet, no changes have been described in the tissues when deficiency of any of the following are produced: chlorine,[31] phosphorus,[32] zinc,[33] and manganese.[34]

On theoretical grounds one might expect that iodine deficiency would lead to cardiac changes, i.e., myxedema. Under stringent laboratory conditions, iodine deficiency is difficult to produce and diets low enough in iodine content have not as yet led to deficient states sufficient to produce severe hypothyroidism.

AMINO ACIDS

Proteins probably constitute the most important group of foodstuffs. Certain of their constituent amino acids can be classed as nonessential, i.e., can be synthesized by the organism, while others are essential and must be furnished preformed in the diet. Deficiencies of all of the essential amino acids have been produced. As yet no striking alterations in the function or structure of the myocardium have been observed.[1]

VITAMINS

At the present time a great deal has been learned about the biologic role of the water-soluble vitamins. These indispensable nutrients function as integral parts of coenzymes which are important in the metabolism of carbohydrates from which the myocardial fibers derive their energy. Thus, thiamine is a part of the coenzyme cocarboxylase which catalyzes the reaction of pyruvate to acetate. It would not appear necessary to detail the functions of other vitamins which will be mentioned below; specific chemical derangements which occur when they are lacking will indicate their metabolic roles. Most of the vitamins to be discussed lead to physiologic disturbances when they are removed from the diet. In only thiamine and biotin deficiencies have morphologic alterations been described.

Thiamine

Although many of the earlier experiments dealing with the effects of thiamine deficiency on the heart are open to criticism because other essentials have been lacking, it seems clear enough that the integrity of the myocardial fiber is dependent on the presence of this vitamin. Such has been unequivocally shown in swine,[35] rats,[36] dogs,[37] foxes,[38] cats,[39] and monkeys,[40] in which physiologic as well as morphologic alterations have been produced.

Among the functional changes, bradycardia was early described as a distinctive feature of vitamin B deficiency in the rat.[41] This observation has been confirmed in more elegant experiments in other species. So, too, electrocardiographic alterations have been reported in

swine.[42] Such changes consist of prolonged P-R intervals, abnormalities, in the P wave, increase in T_4, nodal and ventricular premature beats, A-V dissociation, complete block, and auricular fibrillation.

Sudden death has been a prominent feature of the thiamine deficiency syndrome in swine. Death may or may not be preceded by labored respirations and cyanosis, both made worse by exercise.

At autopsy the heart is generally found to be dilated (rats, dogs, swine), but evidence for hypertrophy is equivocal. Microscopic lesions have now been described in rats,[36] dogs,[37] foxes,[38], and swine.[35] In the latter species alterations have been found after only 37 days on a deficient diet. The change consists of focal or even diffuse necrosis of myocardial fibers. The earliest alteration is loss of striation of the fibers with vacuolization and hyaline change. With necrosis leukocytes appear. On occasion, such areas are large enough to be visible macroscopically. Lesions may be found in both auricles and ventricles, though on one occasion in our own experience alterations were restricted to the auricular myocardium. As might be expected, if death does not ensue, the necrotic foci are replaced by connective tissue fibers.

A great deal of speculation has been directed at the pathogenesis of the cardiac muscle changes. As is well known, excess amounts of pyruvate accumulate when thiamine is not present. Large amounts of sodium pyruvate have, therefore, been administered to normal and thiamine-deficient rats.[41] Only slight changes are found in the heart rate and in the electrocardiographic tracings.

Some time ago we were impressed by the close similarity to the lesions produced by potassium deficiency and those resulting from thiamine deprivation. It was expected that more severe alterations might be found when both essential nutrients were eliminated from the diet. On the contrary and to our surprise, no lesions whatsoever appeared in the myocardium.[43] The reason for this has not as yet been elucidated, though the intimate relationship of potassium and thiamine to carbohydrate metabolism is well established.

There can be no doubt that thiamine deficiency in the human leads to beriberi heart disease. The picture is complicated by the too often unrealized fact that beriberi may not, and undoubtedly usually is not, a disease produced by a deficiency of a single nutrient. Endemic beriberi is associated with a dietary consisting predominantly of milled rice. This foodstuff is also deficient in numerous other essential nutrients in addition to thiamine.[1]

"Beriberi heart disease" has been used in this country, by both clinicians and pathologists, as a sort of catch-all in which to dispose of cases of heart failure which could not be satisfactorily explained on a more common basis. Although we have been interested in this problem for some years, particularly as a result of observations in animals,[35] we have to date never seen a case at autopsy in this country. Nor have we had occasion to study the heart of patients dying of beriberi in the Orient and elsewhere. Consequently, we question the desirability of a discussion of the effects of thiamine on the human heart until more pointed studies, both biochemical and anatomical, can be made.

Pantothenic Acid

In pantothenic acid-deficient ducklings the coenzyme A content of both the auricle and ventricle is reduced as much as 80 per cent.[44] Oxygen consumption and pyruvate utilization of tissue slices are also lowered, but not in proportion to the coenzyme A reduction. As far as we are aware, pantothenic acid deficiency has not been shown to result in anatomical lesions in the heart of the experimental animal.

Pyridoxine

Pyridoxine deficiency, sufficient to produce anemia in rats, can lead to a reduction in transaminase activity in heart tissue homogenates without, however, seriously affecting the overall respiration or the utilization of pyruvate or lactate.[45] Such changes in transaminase activity cannot be demonstrated in slices. No morphologic alterations have as yet been demonstrated in the myocardium of pyridoxine-deficient animals.

Niacin

Nicotinic acid (niacin) is an important part of the coenzymes, tri- and diphosphopyridine nucleotide, which are concerned in glycolysis and tissue respiration. Biochemical alterations might be expected to occur in the myocardium if niacin were present in the diet in reduced quantities. Decreases in the utilization of lactate but not of pyruvate have been described in ducks.[46] No histologic changes have been described in niacin-deficient animals.

Biotin

The oxygen uptake of myocardial slices from biotin-deficient ducks is decreased in the presence of substrates such as succinate or pyruvate.[47] Necrosis of the myocardium has also been described in biotin deficiency.[48]

Riboflavin

This constituent of a number of flavoprotein enzymes has not as yet been shown to affect the metabolism of the myocardium *in vitro*. Recently, however, changes have been observed in the cardiovascular system of the young from mothers fed an antimetabolite, galactoflavin.[49] Such alterations consist of congenital malformations—septal defects, aortic arch anomalies—and appear similar to the changes to be described below under pteroylglutamic acid and vitamin A.[50,52]

Vitamin B_{12}

No physiologic or morphologic changes in the myocardium have yet been described as a result of vitamin B_{12} deficiency.

Pteroylglutamic Acid

Observations similar to those described below under vitamin A deficiency[52] have been reported by Baird and associates.[50] Pregnant females were fed a synthetic diet containing 1 per cent succinylsulfathiazole and the antagonist X-methyl-pteroylglutamic acid plus added pteroylglutamic acid; a deficient state was then produced in the fetus at varying stages of development by removing the pteroylglutamic acid for varying periods of time during gestation, such as at the seventh to the ninth day, the tenth to the twelfth day, etc. One or more cardiac anomalies were found in 130 of 423 fetuses studied. Inasmuch as the cardiac mesoderm is not seen until the eighth day of gestation, changes were not observed until the seventh to ninth day, at which time 28 per cent of the young *in utero* showed changes. The incidence jumped to 57 per cent in the fetuses examined from the ninth to the eleventh days. This high incidence apparently coincides with the development of the first aortic arch on the tenth day of gestation. No changes were seen if the deficiency was produced after the tenth day.

As in the young of vitamin A-deficient mothers, a most prominent malformation was interventricular septal defect, which was seen in 92 of the 130 young showing changes. The other prominent abnormalities were found in the aortic arch derivatives and consisted of: persistent truncus arteriosus, double aortic arch, right aortic arch, absence of ductus arteriosus, absence of the arch, and various patterns in the origin of the subclavian vessels.

These changes are most striking and illustrate the profound effects which a deficiency of a single nutrient may have on the development of an organ system at a critical period of growth. As in vitamin A maternal deficiency, the young of the pteroylglutamic acid-deficient mothers likewise show malformations in other organs, such as the eye, skeleton, nervous system, etc.

Ascorbic Acid

No biochemical alterations in the myocardium have been described in ascorbic acid deficiency. The situation with respect to morphologic changes is a confused one and needs further study.[1] We have called attention to the occurrence of sudden death in infants with scurvy.[51] The cause may have been ascorbic acid deficiency

Vitamin A

No changes in the heart have been described in growing or adult animals when placed on vitamin A-deficient diets. However, extremely interesting and important observations have been reported in the young from vitamin

A-deficient mothers.[52] Such mothers were fed deficient rations prior to and during pregnancy. In 75 per cent of the fetal or newborn rats examined some degree of malformation was found. Ocular and genitourinary anomalies were encountered most often. However, an appreciable number of malformations of the heart and vessels derived from the aortic arch system were also present.

In the heart, two types of abnormality were observed. The first defect was failure of the interventricular septum to close; this left an opening of varying size. The second abnormality consisted of a general failure in development of the myocardium, so that the ventricular walls had a spongy, trabeculated appearance.

Aortic arch anomalies were equally frequent. The basic patterns varied, such as retention of both fourth and/or sixth arches; reversed asymmetry, i.e., right instead of left fourth and sixth arches; supernumerary arches; absence of both fourth and/or sixth arches.

No changes were seen in fetuses before the twelfth day of gestation, so that it was concluded that all of the malformations of the heart and vessels were the result of an interference with growth processes occurring on or following the twelfth day of gestation.

These observations of Wilson and Warkany[52] are most important and open a new chapter in the experimental production and study of congenital cardiac diseases.

Vitamin E

The dystrophic changes which are found in skeletal muscle in vitamin E deficiency are too familiar to require more than mention. It was not unexpected, therefore, to find changes in the myocardium as well. Both physiologic and anatomic alterations have been described in several species. In the rat which has remained on a deficient diet for over a year, necrosis of cardiac muscle fibers was first reported by Mason and Emmel.[53] The damaged muscle fibers are replaced by connective tissue bundles. The changes resemble those already described in potassium or thiamine deficiency save in one respect, that is, the presence of a characteristic pigment, ceroid, in material from animals made deficient in vitamin E.

TABLE II

Effects of Deficiency of Vitamins on the Heart

Vitamin	Functional change	Anatomical change
Thiamine	+(42)*	+(35, 40)*
Pantothenic acid	+(44)	0
Pyridoxine	+(45)	0
Niacin	+(46)	0
Biotin	+(47)	+(48)
Riboflavin	—	F† (49)
Pteroylglutamic acid	—	F† (50)
Ascorbic acid	—	? (1)
Vitamin A	—	F† (52)
Tocopherols	+(54, 55)	+(53, 54, 56)

* Numbers in parentheses refer to reports listed in References.

† Fetus.

More pronounced alterations have been described in rabbits placed on vitamin E-deficient rations.[54] Here electrocardiographic tracings in some revealed elevation of the S-T segment and inversion of the T wave in lead II. Microscopic examination of the myocardium indicates changes similar to those which have been found in skeletal muscle. There is first necrosis with loss of striations; next, nuclear changes, i.e., pyknosis and karyorrhexis; these, then, are followed by an inflammatory reaction. Calcification of some of the fibers is described. The lesions tend to be more extensive when the fat content of the diet is high. The effect of treatment in these animals has not been investigated.

In addition to the rat and rabbit, changes have been reported in calves on vitamin E-deficient rations. The first report by Gullickson and Calverley[55] dealt with animals which died suddenly. Electrocardiograms four months prior to death revealed an increase in the P-R interval which was followed by changes in the QRS complex. Histologically, atrophy and scarring of the ventricular wall were briefly described. More recently, Blaxter and co-workers[56,57,58] have described in more detail the changes which may be encountered in the calf.

TOTAL AND PARTIAL STARVATION

Experiments in animals and observations in humans subjected to varying degrees of malnu-

trition have revealed profound physiologic and anatomic alterations in the heart. The literature on the heart and on circulatory function in malnutrition has been summarized in a now classical monograph, *The Biology of Human Starvation* by Ancel Keys and his co-workers.[59]

There are numerous references to evidence that caloric restriction leads to certain functional changes: bradycardia, fall in blood pressure with weak pulse, decreased cardiac output, increase in circulation time, and decrease in venous pressure. Careful studies at the University of Minnesota have placed these clinical observations on a sound basis. The total loss of weight over 24 weeks of semi-starvation was approximately 25 per cent of the initial weight in these experiments. The minimum average pulse was 35.3 beats per minute. The blood pressure decreased from an average of 106.8/69.7 to 98.6/62.7 mm Hg. The venous pressure decreased by over half its normal value. The work of the heart was calculated as half the control value. Teleoroentgenograms revealed a decrease in the size of the heart.

That actual weight of the heart is decreased in starvation has been known for some time. In German concentration and prison camps the average heart weight of starved persons was 200–220 grams, or a decrease of 25–30 per cent of normal. Studies on 492 adult individuals dying in the Warsaw Ghetto revealed heart weights ranging from 275 to 220 grams, or an average of 20 per cent decrease in size. Such changes in the human have a counterpart in the experimental animal (rat) where in complete starvation plus water the heart weight decreases 19.8 per cent while the body weight is decreased 26.9 per cent.[59]

Histologic observations on both humans and experimental animals have not shown very much save the presence of pigment in the muscle fibers, i.e., classical "brown atrophy," although sometimes excess fat may be observed. This phase certainly deserves further study, particularly in easily available experimental animals.

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Research 1956

"The achievements of science in some fields have caught the popular imagination of the public to a degree that has never occurred before. The press, radio, and television continually remind its readers, listeners, or viewers that given enough money the "scientists" can solve any problem and if given twice as much money can do it in half the time. We have seen this doctrine promulgated to the point where the ordinarily citizen can hardly but the simplest necessities of life without the oft repeated assurance that its taste, palatability, and nutritive qualities have been assured by the research efforts of a devoted, unbiased, and highly skilled corps of scientists. Of even graver import is the persuasion of the public that the donation of large sums of money is all that is needed to eradicate or cure the more serious diseases of mankind. I am disturbed by the implied promises in these annual compaigns for several reasons; first, because the present lines of research may not succeed in living up to such promises, leaving the public, upon whom the responsibility for the support of future research must depend, with such a sense of disappointment, if not worse, that the slow but steady accretion of basic knowledge will be retarded; second, because this is not the right form of public education as to how research is done, and by whom it is done; third, because it relegates to a secondary place the vastly more important task of finding and training new investigators, not as junior members in a team seeking to find a cure for a particular disease, but as independent and creative investigators in their own right; fourth, because research in a few areas is overemphasized and overfinanced, leaving other areas, the future importance of which no one can estimate, understaffed and lacking the often modest requirements for work, and lastly, because support being on a yearly basis does not allow the unhurried contemplative study of the problem that is essential, nor does it give the degree of security that such efforts require."

—C. N. H. Long, *Pediatrics* 15: 204, 1955.

Serum Cholesterol Concentration in New-Born African and European Infants and their Mothers

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THAT serum cholesterol concentrations are lower in the African than the European is being borne out by increasing evidence.[1–4] Thus, at this center, we have found the mean cholesterol level in 70 healthy South African European subjects of both sexes (age group 21–30 years) to be 215 mg, per 100 ml, while a group of 89 African nurses of similar age had a mean cholesterol level of 174 mg per 100 ml. A group of 45 male Bechuana Africans (age 21–35 years) had a mean cholesterol level of 156 mg per 100 ml.[5] These figures closely resemble those of Walker and Arvidsson[4] on similar groups of subjects. We found no statistical differences between mean values of comparable age groups of the sexes. The question arises: Are lower cholesterol levels characteristic of the African and apparent at birth, or are mean values in the new-born the same for both racial groups?

In a previous publication, we demonstrated that serum proteins and certain liver function tests in the umbilical cord bloods of European and Bantu infants showed no statistical differences, whereas the mothers of the two racial groups showed marked differences.[6] Since data on serum cholesterol were also determined at the same time, and in view of the present interest in the role of serum cholesterol concentration in atherogenesis, we have thought it worth while to present and discuss our results.

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SUBJECTS AND METHODS

Subjects

The subjects, 37 European and 51 African mothers, were patients at Queen Victoria Hospital for Women (149 beds) and Baragwanath Non-European Hospital, Johannesburg (1350 beds). Age ranges and mean ages of European and African mothers were 18–42 (mean 25) and 18–40 (mean 26) years, respectively (Table I). All mothers were healthy

TABLE I

Age Distribution of African and European Mothers

Years	African	European
16–20	5	8
21–25	21	14
26–30	17	9
31–35	5	4
36–40	3	1
41–45	..	1
Total	51	37

and showed no signs of undernourishment. Where ante- or post-partum hemorrhage, toxemia, hypertension, edema, diabetes, or premature labor were featured, the mothers, as well as their infants, were excluded from this study. Furthermore, infants with a birth weight below $5^1/_2$ lb, together with their mothers, were also excluded. The blood from the infants was drawn from the placental end of the umbilical cord, after division, immediately after pulsation had ceased. The mothers' blood was obtained from the cubital vein with the minimum of stasis and was collected before or after labor, but always within 24 hours of parturition and usually within 12 hours.

Methods

Serum cholesterol determinations were carried out as described by Kaye[7] with minor modifications. Results obtained by this method were compared with Sperry and Webb's modification of the Schoenheimer and Sperry technique.[8] Our mean results for total and free cholesterol were 5 per cent and 4 per cent higher, respectively, than those obtained using the latter technique.

RESULTS

The results are summarized in Tables II–IV. Table II summarizes the results of total cholesterol levels. The serum cholesterol concentration was 71.3 mg per 100 ml in the African new-born infant and 75.3 mg per 100 ml in the European infant, there being no statistical differences between the two racial groups. The mean cholesterol concentration was 189.8 mg per 100 ml in the African mother and 258.5 mg per 100 ml in the European mother, a very highly significantly lower value in the African (P = 0.000001).

Table III summarizes the concentrations of free and esterified cholesterol and, here too, the African and European infants showed no statistical differences; but highly significant differences were found in the maternal groups. The ratios of cholesterol esters to total cholesterol are summarized in Table IV. No differences in the ratio were found between the European or African groups. The ratios obtained in both the infants and the adults were within the range of normality (70–75 per cent).[9]

TABLE II

A Comparison of the Total Serum Cholesterol Levels of African and European Infants and Their Mothers

	Infants		Mothers	
	African	European	African	European
mg/100 ml				
31–50	6	6	—	—
51–70	21	15	1	—
71–90	15	12	—	—
91–110	5	9	2	—
111–130	3	2	1	—
131–150	—	—	3	1
151–170	—	—	9	—
171–190	—	—	15	4
191–210	—	—	5	2
211–230	—	—	7	1
231–250	—	—	5	6
251–270	—	—	—	7
271–290	—	—	1	8
291–310	—	—	—	4
311–330	—	—	1	2
331–350	—	—	1	—
351–370	—	—	—	1
371–390	—	—	—	1
Total	50	44	51	37
Means	71.30	75.27	189.81	258.47
S.D.	19.29	21.05	49.79	50.13
D_m/S.E.d.	0.95		6.36	
"P"	0.34		0.000001	
Significance of "P"	Not significant		Very highly significant	

DISCUSSION

This investigation shows that the mean cholesterol level in the African adult is significantly lower than that of a similar group of European subjects, whereas no significant difference exists between the cholesterol levels of African and European new-born infants. Furthermore, it is clear that the ratio of cholesterol esters to total cholesterol does not differ in the new-born and adult subjects of both racial groups. Regarding the latter findings, it is interesting to note that in Sperry's investigation,[9] the ratio of esterified to total cholesterol is lower in infants (41–72 per cent) than in adults (70–75 per cent); he considered that the mechanism whereby the proportions of total and free cholesterol in the adults is maintained within narrow limits is not yet fully established in the neonatal period. In addition, Boyd,[10] in an analysis of 29 samples of plasma in the new-born, showed that the ratio of esterified to total cholesterol was 59 per cent (mean total cholesterol 34 mg, cholesterol esters 20 mg, free cholesterol 14 mg per 100 ml) as against a mean of 71 per cent in adults. In the plasma of one infant, there were no cholesterol esters. This author stated that there was a greater variation in the infant than in the adult, and among the possible causes for the lowered percentage of esterified cholesterol in infants were listed the state of nutrition of the new-born child, the effects of

TABLE III

A Comparison of the Free Cholesterol and Cholesterol Esters of African and European Infants and their Mothers

	Free cholesterol				Cholesterol esters			
	Infants		Mothers		Infants		Mothers	
	African	European	African	European	African	European	African	European
mg %								
1–20	26	24	—	—	—	—	—	—
21–40	23	20	14	5	12	9	—	—
41–60	1	—	26	5	25	15	1	—
61–80	—	—	11	20	10	17	2	—
81–100	—	—	—	3	3	3	2	—
101–120	—	—	—	3	—	—	7	1
121–140	—	—	—	1	—	—	13	2
141–160	—	—	—	—	—	—	14	6
161–180	—	—	—	—	—	—	6	6
181–200	—	—	—	—	—	—	3	6
201–220	—	—	—	—	—	—	1	8
221–240	—	—	—	—	—	—	—	2
241–260	—	—	—	—	—	—	1	5
261–280	—	—	—	—	—	—	1	1
Total	50	44	51	37	50	44	51	37
Means	20.1	19.818	48.833	67.392	52.10	56.636	142.167	191.17
S.D.	7.54	8.090	12.629	22.03	12.42	17.47	38.53	23.08
D_m/S.E.d.	0.186		4.6		1.43		7.431	
"P"	0.8		0.0001		0.15		0.0000001	
Significance of "P"	No		Highly		No		Very highly	

TABLE IV

Serum Cholesterol Ratio. A Comparison of the Ratio of Cholesterol Esters to Total Cholesterol in African and European Infants and Their Mothers

	Infants		Mothers	
	African	European	African	European
Mean total cholesterol, mg per 100 ml	71.3	75.27	189.81	258.47
Mean cholesterol esters, mg per 100 ml	52.1	56.63	142.16	191.17
Ratio of esterified to total cholesterol, %	73	75	75	74

anesthesia and analgesics given during labor, whether labor was easy or difficult, and the possibility of fasting of the mother during labor having influenced the lipid content of the newborn. Our findings, therefore, of no significant difference in the percentage of esterified cholesterol to total cholesterol between infants and adults of both racial groups stands in marked contrast to the data of the workers cited. At present, we have no satisfactory explanation.

The reason for the serum cholesterol level of the African adult being lower than that of the European is open to speculation; probably no single factor is responsible, but rather a combination of factors, some recognized and others still unknown. Among the possible reasons are differences in race, in the nature of the diet consumed and the relevant pattern of metabolism thereby imposed, in the incidence of hepatocellular disease and imbalance of sex hormones. These aspects will now be briefly considered.

Racial Differences

There is no evidence that serum lipids are influenced by race.[11] Moreover, as Beyers and associate workers maintain, food habits of various races are probably far more responsible for differences in cholesterol concentration than intrinsic racial peculiarities.[12] Our finding that at birth there is no difference in cholesterol concentration in European and African groups strongly supports the view expressed by various

investigators[4,11,12] that the racial factor is not relevant to the problem.

Diet

The diet of the African differs in many respects from that of the European. Thus, as has been recently pointed out, although the African probably does not suffer severely from calorie deficiency, the average diet consumed is low in animal protein and in certain vitamins and mineral salts, but is high in carbohydrate intake and crude fiber. The fat intake is low, usually less than 20 per cent of the total calories being provided by dietary fat.[4,13] In contrast, the proportion of calories supplied by fat in local European diets is probably 35–40 per cent.[14] Keys and associates, who have carried out exhaustive studies in this field, consider that, although the serum cholesterol level is subject to a complex regulation which is affected by many factors, the clearest of these is the diet, and the major dietary factor is the fat/calorie ratio.[15–19] In other words, the higher the fat intake the higher the serum cholesterol concentration and vice versa. Although Walker and Arvidsson have demonstrated a correlation between fat intake and serum cholesterol concentration in the African,[4] it is quite obvious, as these workers maintain, that fat intake is unlikely to be the only or even the most important influencing factor. There is now ample evidence, for example, that the true Eskimo, although subsisting on a high fat diet, does not show elevated cholesterol levels, and apparently the incidence of severe atherosclerosis is not high.[20–23] Moreover, although Keys and co-workers do not consider the distinction of animal versus vegetable fat to be of peculiar importance,[15–19] it is becoming increasingly apparent that "pure" vegetarians consuming a high vegetable fat intake are marked by cholesterol levels lower than those of omnivorous eaters.[24–25] At the present stage of knowledge, we agree with Walker[13] that it would be premature to single out one feature of the Bantu diet, namely, low fat intake, as being wholly or even mainly responsible for the low serum cholesterol levels observed.

An explanation to be considered for the relatively low level of serum cholesterol found in the African is the possibility that maize, being the staple food of the African, and constituting a high proportion of his diet, may contain a substance, in the nature of an antimetabolite, interfering or competing with the absorption of cholesterol from the bowel in a manner similar to that previously shown to occur in respect of certain vegetable fats[26,27] and of sitosterol. This hypothesis warrants further study.

Furthermore, it is well to realize that our knowledge of the metabolism and fate of cholesterol, whether ingested or synthesized, is far from complete. We know very little of the factors which influence the rate of cholesterol synthesis, its absorption and re-absorption, destruction by intestinal microflora, or excretion in the feces. Certainly it is conceivable that the processes are not the same for different types of diets. In addition, the effect of the superimposition of malnutrition and of undernutrition on any particular type of diet also requires consideration. The vast gaps in our information underline the caution that must be exercised in crediting any particular dietary constituent with undue etiological significance in atherogenesis.

Liver Disease

In an investigation still in progress, we have found that approximately 60 per cent of the adult Bantu population examined show biochemical evidence of liver derangement, believed to be due to the effects of chronic malnutrition.[30] Byers *et al.*[12] considered that the liver is the only organ supplying significant amounts of endogenously-derived cholesterol to the plasma. On account of our observation that more than half of the Bantu population show evidence of abnormal liver function, the question arises as to whether a lowered rate of cholesterol synthesis due to chronic liver disease may not be responsible for the lowered cholesterol concentration found in the African. In this respect Walker and Arvidsson,[4] investigating a group of Bechuana and Basuto Africans, 67 per cent of whom showed marked biochemical evidence of liver damage, found no significant difference in mean serum cholesterol

in those showing abnormal liver function tests compared with those who showed slight or no evidence of liver disease. They reported a similar situation occurring in a Bantu group on a partially European diet. In liver disease, hypocholesterolemia is only found in the presence of severe hepatic damage, and even in acute hepatitis and portal cirrhosis it is uncommon to find low cholesterol concentrations. However, in mild to moderate degrees of liver damage, although the total cholesterol concentration is not usually low, the degree of esterification of cholesterol is usually disturbed at an early stage, and is indicative of hepatocellular damage.[31] In the present investigation, however, we found no reduction in the percentage of esterified cholesterol, and this, despite the high incidence of liver disturbances in the Bantu mother.[6] Thus, it would appear unlikely that liver damage in the African directly affects their metabolism of cholesterol, but at the same time, the implication of hormonal imbalance due to a malfunctioning liver must be considered as a possible contributory factor.

Sex Hormones

The liver exerts an important influence on hormonal metabolism in maintaining the delicate balance between estrogens and androgens. In chronic liver disease, disturbance of sex hormone balance is encountered. Thus, menstrual disturbances occur in the female, and gynecomastia, testicular atrophy, loss of pectoral and axillary hair, loss of libido, impotence and sterility are observed in the male. Gynecomastia and testicular atrophy are more common in Africans than in Europeans, and Gillman and Gillman[32] have alluded to the correlation between gynecomastia and atrophy of the testis, on the one hand, and liver damage, on the other. These authors, however, state that they were unable to correlate the presence or absence of gynecomastia with the extent or severity of the pathologic changes in the liver. The incidence of gynecomastia in their series was only one sixth that of moderate or severe liver damage. It is known that in chronic liver damage, increased estrogen blood levels are found, as well as decreased androgen levels, which in turn may be due to testicular atrophy.[33] Eilert[34] has shown that increased estrogen levels depress plasma cholesterol; and Boyle[35] has reported reduction in all serum lipids, including cholesterol, in humans during long-term administration of estrogens. Thus, alterations in the balance between the male and female sex hormones, arising from chronic liver disease, may well be involved in the observed difference between the cholesterol levels in African and European subjects.

Much further work is required before the lowered cholesterol concentration in the African can be adequately explained. The question of reaction to stress in the African, the effects of nutritional disturbances on the thyroid, adrenal, and other endocrine glands, and the role of the steroid hormones in cholesterol metabolism, all still require much further investigation before a definite answer can be given to the problem. The cholesterol problem, moreover, seems to be very intimately related to the present high increasing incidence of atherosclerosis and coronary heart disease. In the Africans we have ideal material for a study of this whole subject, since we have a racial group (living side by side with the European population) in whom severe coronary disease is almost non-existent and who normally have low serum cholesterol values.

SUMMARY

Serum was collected from 51 African and 37 European mothers and their infants and determinations were made of total and esterified cholesterol concentration. It was found that: (1) mean cholesterol concentrations of infants from both groups were identical; (2) mean cholesterol concentrations in the African mothers were significantly lower than among the European mothers; and (3) no significant difference was found in the percentage of esterified serum cholesterol in the infant and maternal groups from both races.

The possible parts played by race, diet, liver disease, and sex hormones, in producing low serum cholesterol concentrations in African adults, are discussed.

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Climate + Intake = Temperament (?)

"Huge white bodies, cool-blooded, with fierce blue eyes, reddish flaxen hair; ravenous stomachs, filled with meat and cheese, heated by strong drinks; of a cold temperament, slow to love, home-stayers, prone to brutal drunkenness: these are to this day the features which descent and climate preserve in the race. . . . There is no living, in these lands, without abundance of solid food; bad weather keeps people at home; strong drinks are necessary to cheer them; the senses become blunted, the muscles are braced, the will vigorous."

—H. A. Taine. *History of English Literature:* The Saxons, 1863.

The Strenuous Pleasures of Barbarians—and Others

"If the carnivorous, warlike, drinking savage, proof against the climate, still shows beneath the conventions of our modern society and the softness of our modern polish, imagine what he must have been when, landing with his band upon a wasted or desert country, and becoming for the first time a settler, he saw extending to the horizon the common pastures of the border country, and the great primitive forests which furnished stags for the chase and acorns for his pigs. The ancient histories tell us that they had a great and coarse appetite. Even at the time of the Conquest the custom of drinking to excess was a common vice with men of the highest rank, and they passed in this way whole days and nights without intermission. Henry of Huntingdon, in the twelfth century, lamenting the ancient hospitality, says that the Norman kings provided only one meal a day, while the Saxon kings used to provide four. . . . To shout, to drink, to gesticulate, to feel their veins heated and swollen with wine, to hear and see around them riotous orgies, this was the first need of the Barbarians. The heavy human brute gluts himself with sensations and with noise."

—H. A. Taine. *History of English Literature:* The Saxons, 1863.

Intestinal Absorption and Hepatic Uptake of Radioactive Vitamin B_{12} in Various Age Groups and the Effect of Intrinsic Factor Preparations

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RECENT studies on the absorption and fate of vitamin B_{12} administered parenterally or orally to individuals over 60 years of age appear to reveal a number of agewise differences as compared to young individuals. A larger portion of vitamin B_{12} injected intramuscularly is retained by elderly subjects than by the young.[1,2] The mean vitamin B_{12} serum levels of the young appear to be significantly higher than those in the older age group.[2] Finally, elderly people show a much less marked elevation of the vitamin B_{12} blood levels than do young subjects after the administration of a standard oral dose of vitamin B_{12}.[3] On the basis of these data, Chow and associates[2,3] concluded that older people are unable to absorb exogenous vitamin B_{12} from the intestine as well as young persons, possibly due to a lack of intrinsic factor from the stomach; this would result in a lowering of vitamin B_{12} levels in blood and presumably also in tissues.

The work of other investigators does not seem to corroborate these inferences. Intestinal absorption of radioactive vitamin B_{12} in various age groups, studied with the use of Schilling's urinary excretion technique,[4] indicates no statistically significant differences between young and old people.[5] In discussing these findings Krevans[6] reported that his data, obtained by means of a fecal excretory technique of Heinle *et al.*,[7] harmonize with data of Schilling, in so far as old people do not excrete more radioactive vitamin B_{12} in the feces than young subjects after oral administration of a standard dose of radioactive cobalt-labeled vitamin B_{12}. Most recently, Swendseid and co-workers[8] reported that the amounts of vitamin B_{12} stored in the liver of both young and old did not differ significantly when determined microbiologically *post mortem*. However, since it was uncertain how many of these subjects, prior to their death, received injections of vitamin B_{12} (which can be stored for months and probably years in the liver), possible differences in many of these cases could have been masked. On the other hand, unpublished data of Boger[9] on blood levels of vitamin B_{12} revealed a statistically significant regression of vitamin B_{12} blood levels in line with the advancing age in women; his data obtained in men appear to be less conclusive.

In view of the importance of vitamin B_{12} in human nutrition, especially in regard to the metabolism of nervous tissue and hematopoietic organs, the existence of a possible defect in intestinal absorption of vitamin B_{12} in aged people might have great importance for the understanding of some gerontological problems. The contradictory results reported in the literature induced us to re-investigate this problem with other techniques and from some other points of view. The present study was undertaken to determine whether or not there is an intestinal absorption defect in regard to

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vitamin B_{12} in aged people, and whether this defect, if present, is due to the absence of Castle's intrinsic factor from the stomach, or whether it depends on an inherent absorption defect of the small intestine itself due to aging. It seemed also of interest to determine whether or not the oral administration of intrinsic factor preparations could enhance the intestinal absorption of vitamin B_{12} among subjects in the old age group, and what was the relationship of this effect to the secretory status of the stomach.

MATERIAL AND METHOD

Intestinal absorption of vitamin B_{12} was evaluated by measurement of hepatic uptake of cobalt-labeled vitamin B_{12}, according to our technique.[10,11] The hepatic uptake method is based on determination of the increment in the radioactivity counts over the liver following the oral administration of 0.5 μg of radioactive vitamin B_{12} of high specific activity, 770 μc/mg, i.e., containing 0.385 μc. Co^{60}. The counts were taken with the scintillation counter, provided with a NaI-thallium crystal 1 inch in diameter and $^1/_2$ inch in depth at 1000 volts without collimator, in direct contact with the skin over the anterior, antero-lateral and mid-lateral projections of the liver on the sixth and seventh day after ingestion of radioactive vitamin B_{12}, with the patient in a strictly supine position and with the tube perpendicular to the skin. We used a scaler with amplifier, preset time, and precise (helicoid) regulation of the high voltage and sensitivity. Three to four projections from other areas of the lower, mid, and left abdomen were used as controls to rule out the occasional interfering retention of radioactive material in the intestine. The uptake of radioactivity over the liver in counts per minute was averaged from all three liver projections on both days of assay, calculated above background (equal to 70–90 counts per minute), and corrected for the efficiency of scaler and decay of Co^{60} with the use of dried Co^{60}-vitamin B_{12} standard; the final figures were then calculated per 1 μc dose of Co^{60}.

The actual investigation was done on 60 individuals ranging from 18 to 90 years of age. The age distribution is shown in Table I. In the younger age control group (below 60) there were 9 men and 8 women, in the elderly group, 14 men and 29 women. One-half of the patients were seen at the medical clinics and wards of Flower and Fifth Avenue Hospitals; the other half were from Bird S. Coler Memorial Hospital and Home. None of these 60 subjects was anemic, and none had an organic disease of the digestive tract or the liver. Several of the subjects had one or another kind of the respiratory, circulatory, or arthritic disorders common to the elderly population, the nature of which, however, was rather irrelevant to the problem studied. The investigation was done under uniform conditions. The same equipment and the same cobalt-labeled vitamin B_{12} preparation was used throughout the study. During the test period the patients did not receive any medication which could have interfered with the intestinal absorption of vitamin B_{12}; special care was taken that no antibiotics, hematinics, vitamins, or laxatives were given.

TABLE I

Hepatic Uptake of Co^{60}-labeled vitamin B_{12} Following Oral Administration of a Standard Dose of Radioactive Vitamin B_{12} in 60 Individuals of Various Age Groups

Age groups		Hepatic uptake of Co^{60}-B_{12} in counts/min/μc Co^{60}		
Age yr	Number of cases	Range	Mean and standard deviation	Median
18–35	8	165–1147	638 ± 331	600
43–58	9	208–761	504 ± 188	523
60–69	14	0–1011	499 ± 265	448
70–79	14	58–874	481 ± 203	506
80–90	15	34–1227	554 ± 299	502
18–90	60	0–1227	528 ± 268	483

Gastric acidity was determined in 21 subjects by the urinary resin exchange test after caffeine test meal; in 15 cases by gastric analysis after histamine; and in 5 cases by both methods simultaneously. The resin exchange test was performed by the method of Segal and co-workers[12], as reported before.[13]* The gastric aspirations were continued for one hour with 15-minute collections following injection

* These determinations were supported by the Sophie D. Cohen and William W. Cohen Foundation

of 0.5–0.6 mg histamine phosphate; the free and total acidity were determined in a routine way with the use of Topfer's reagent and phenophthaleine.

in a dose of 100 mg,* and in 21 subjects we used the intrinsic factor preparation supplied by Organon Inc.,† in a dose equivalent to that contained in 1 or 2 U.S.P. units of oral anti-

HEPATIC UPTAKE OF $Co^{60}B_{12}$ IN COUNTS PER MINUTE PER 1 μC Co^{60} IN 60 INDIVIDUALS OF VARIOUS AGE GROUPS.

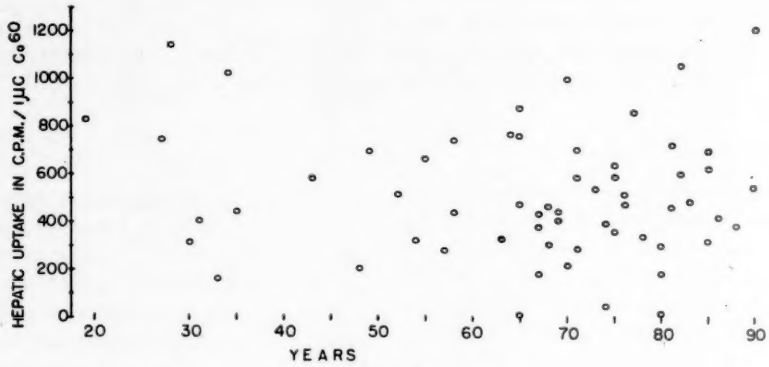

Figure 1

HEPATIC UPTAKE OF $Co^{60}B_{12}$ IN COUNTS PER MINUTE PER 1 μC Co^{60} IN 21 NORMOACID AND 19 HYPOACID OR ANACID INDIVIDUALS OF VARIOUS AGE GROUPS, INCLUDING 30 SUBJECTS OVER 60 YEARS OF AGE.

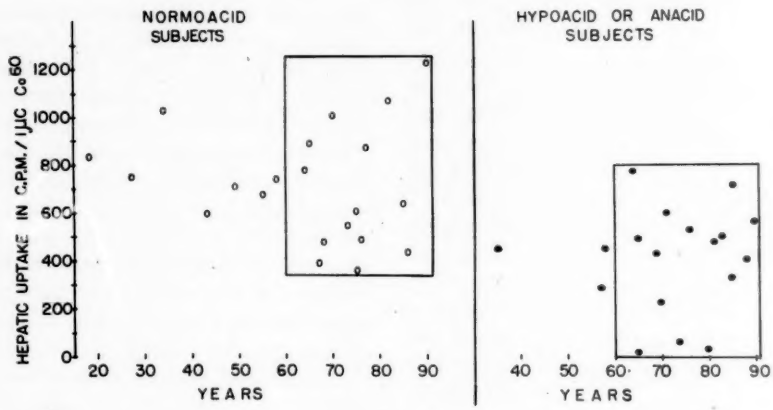

Figure 2

The hepatic uptake was determined in each of the 60 patients following the oral administration of 0.5 μg of radioactive Co^{60}-vitamin B_{12}, and in 44 of these individuals the test was also repeated after the addition of a potent intrinsic factor preparation to the same oral dose of the vitamin. In 30 patients we used a preparation supplied by Ayerst Laboratories pernicious anemia preparation as described below. The intrinsic factor preparations used in this study did not contain any vitamin B_{12}.

* Generously supplied by Drs. John B. Jewell and S. H. Jaros of Ayerst Laboratories, New York, N. Y.

† Generously supplied by Dr. Kenneth W. Thompson of Organon, Inc., Orange, New Jersey.

In seven instances tests were performed with the use of both intrinsic factor preparations one after another, or the same preparation at different doses. Altogether 118 tests were performed on 60 individuals.

RESULTS

Hepatic Uptake of Radioactive Co^{60}-Vitamin B_{12} in Various Age Groups

Figure 1 represents a scatter diagram of the hepatic uptake of 60 individuals of various age groups following oral administration of a standard dose of 0.5 μg of radioactive vitamin B_{12}. No obvious regression of hepatic uptake of Co^{60} as a function of advancing age is apparent from this figure.

The hepatic uptake of Co^{60}-labeled vitamin B_{12} as reported before,[14] varies in a wide range from subject to subject even in normal young individuals. In 17 control patients (age 18 to 58), the hepatic uptake varied from 165 to 1147 counts per minute per μc Co^{60}, with a mean value of 567 counts per minute per μc Co^{60}. In 8 of these 17 patients, the gastric acidity was normal and the hepatic uptake ranged from 208 to 1029 counts per minute. In 3 patients with gastric anacidity or hypoacidity the hepatic uptake ranged from 287 to 452 counts per minute per μc Co^{60}. In 6 subjects of this group acidity was not determined.

When the control group was split into two equal groups of patients below and above forty years of age the mean value of the hepatic uptake in the younger group equaled 638 counts per min/μc Co^{60}, and in the group above forty years of age, 504 counts per min/μc Co^{60}.

In 43 elderly patients over 60 years of age the hepatic uptake varied from 0 to 1227 counts per minute per μc Co^{60}, with a mean value of 513 counts; this is about 10 per cent less on the average than in the younger control group. When this group was split into decades (60 to 70, 70 to 80, and 80 to 90 years of age) the lowest hepatic uptake on the average was observed in the groups of 60 to 80 years of age; whereas the oldest group (above 80) showed much higher mean figures of the uptake of radioactive vitamin B_{12}. The median and the mean values calculated with the standard deviations for each age group are listed in Table I.

Hepatic Uptake of Radioactive Co^{60}-B_{12} and Gastric Secretory Function

In 41 individuals we correlated the hepatic uptake of Co^{60}-vitamin B_{12} with gastric secretory function as determined by the output of the hydrochloric acid in the stomach. The data obtained are shown in Figure 2. It appears that in the group of individuals over the age of 60, the hepatic uptake of radioactive vitamin B_{12} was frequently lower in subjects having gastric hypo- or anacidity, than in those who had normal gastric acidity. The statistical evaluation of these data is presented in Table II. It is evident that the difference between the groups was statistically highly significant with a P value below 0.02, and that the mean values of hepatic uptake in these two groups were 447 and 688 counts per minute per μc Co^{60}, respectively. The range of values of hepatic uptake encountered in normoacid subjects of the older age group was also higher than that observed in subjects with gastric hypo- or anacidity of the similar age. About one-third of the individuals in the normoacid group had a hepatic uptake exceeding the upper limit of values observed in the hypo- or anacid group, and conversely, about one-third of the cases in the hypo- or anacid group had a hepatic uptake below the lower limit of values observed in normoacid individuals (Fig. 2).

TABLE II

Statistical Evaluation of the Hepatic Uptake of Co^{60}-labeled B_{12} in a Group of 30 Subjects Over 60 Years of Age

Gastric secretory pattern	Number of cases	Hepatic Uptake in counts/min/μc Co^{60}	
		Range	Mean and standard deviation
Normoacidity	14	377–1227	688 ± 241
Hypoacidity or anacidity	16	0–780	447 ± 247

Difference between the means	241
Standard error of the difference	92
"t"	2.61
P	0.02

Effect of Intrinsic Factor Preparations upon the Hepatic Uptake of Radioactive Vitamin B_{12}

The data obtained in 44 subjects are given in Table III. In eight of these patients the tests with intrinsic factor preparations were repeated twice, using either both preparations one after another, or the same material at various dosages. Gastric acidity was determined by gastric analysis or by urinary resin exchange test in 30 of the 44 subjects. Three-quarters of the subjects studied belonged to the elderly age group (60 to 90 years of age) with equal distribution among three old age decades (Fig. 3). One-fourth of the subjects were between 30 and 60 years of age.

For the purpose of evaluating the effect of intrinsic factor preparations upon intestinal absorption of vitamin B_{12} we have set the following arbitrary criteria: the intrinsic factor effect was considered significant when the hepatic counts increased or decreased at least 100 counts per minute per μc Co^{60} from the initial level, and when the increase or decrease amounted to at least 25 per cent of the initial value. Both these criteria had to be satisfied before the change was accepted as significant. This was in conformance with our experience on limits of accuracy of the method.

With these criteria the evaluation of the results show that out of 44 tests, the change was not significant in 20; in 4 a definite decrease in hepatic uptake was obtained; and in 20 a significant increase in hepatic uptake followed the administration of intrinsic factor together with radioactive vitamin B_{12}. The comparison of the values of the hepatic uptake of radioactive vitamin B_{12} before and after addition of intrinsic factor preparation shows that while the mean and median hepatic uptake after the administration of radioactive vitamin B_{12} alone was, respectively, 471 and 451 counts per minute per μc Co^{60}, the mean and median hepatic uptake after addition of intrinsic factor preparation was much higher, averaging, respectively, 555 and 576 counts per minute per μc Co^{60}.

Among 20 patients in whom a significant increase of hepatic uptake of radioactivity following administration of intrinsic factor was noted, 13 belonged to the group over 60 years of age (Fig. 3), and the age of the remaining 7 was between 30 and 60 years. This indicates that the addition of intrinsic factor preparation improves intestinal absorption of vitamin B_{12} not only in the elderly, but also in many of the younger age group. One of these seven younger individuals had gastric anacidity, in one gastric acidity was normal, and in five it was not determined. Of 13 cases in the older age group the acidity was normal in three instances and was not determined in one. In six others complete anacidity and in three marked hypoacidity was observed.

There were four patients in whom a significant *decrease* in hepatic uptake of radioactive vitamin B_{12} was found following the addition of a preparation of intrinsic factor. In three acidity was normal, and in one gastric acidity was not determined. This may be contrasted with the 14 patients in whom a significant *increase* in hepatic uptake was observed after the addition of intrinsic factor and in whom acidity was determined; in this group 10 out of 14 cases had anacidity or marked hypoacidity. Finally, of 20 patients with no significant change after the addition of intrinsic factor, in seven hypo- or anacidity was found, and in three it was normal. In seven acidity was not determined.

The statistical evaluation of the relationship of gastric secretory function to the hepatic uptake of radioactive vitamin B_{12} following addition of intrinsic factor preparation is summarized in Table IV. It can be seen that in the group of 17 individuals with gastric hypo- or anacidity a statistically significant increase in hepatic uptake of Co^{60}-vitamin B_{12} followed the addition of intrinsic factor preparation, with a P value below 0.05. The addition of intrinsic factor preparation to vitamin B_{12} in 13 subjects having normal acidity did not cause any significant change in the hepatic uptake.

DISCUSSION

It appears (Fig. 1) that there is no evidence of a general regression of hepatic uptake of radioactive vitamin B_{12} as a function of advancing age. Because the hepatic uptake reflects the extent of vitamin B_{12} absorption

TABLE III

Hepatic Uptake of Co^{60}-labeled Vitamin B_{12} Following Oral Administration of a Standard Dose of Radioactive Vitamin B_{12} Alone and Together with Intrinsic Factor Concentrate

No.	Sex	Age	Diagnosis	Free HCl in gastric juice*	Hepatic uptake of Co^{60}-B_{12} in counts/min/μc Co^{60}			
					After Co^{60}-B_{12} alone	After addition of intrinsic factor to Co^{60}-B_{12}		
1	F	30	No disease	—	321	382	0–1†	
2	F	31	Anxiety neurosis	—	410	769	0–1	
3	M	33	No disease	—	165	330	0–1	
4	F	35	Spontaneous hypoglycemia	D	452	536	Ay§	
5	M	48	Irritable colon	—	208	566	0–1	
6	M	49	Ulcer syndrome	N	707	923	Ay	
7	M	52	Parkinsonism	—	523	648	0–2‡	548 Ay
8	F	54	Essential hypertension	—	330	604	0–1	
9	F	55	Secondary iron-deficiency anemia	N	679	634	Ay	
10	M	58	Hemiplegia	N	761	557	Ay	
11	F	58	Hemiplegia	A	450	903	Ay	
12	F	63	Bundle branch block	—	340	395	0–1	
13	M	64	Hemiplegia	A	780	992	Ay	
14	F	65	Hemiplegia; diabetes	D	484	880	Ay	
15	F	65	Hemiplegia; arteriosclerotic heart	D	0	58	Ay	
16	M	67	Rheumatoid arthritis	N	394	352	Ay	
17	F	67	Hypertensive heart disease	—	443	226	0–1	246 0–2
18	F	68	Osteoarthritis	—	325	391	0–1	
19	M	68	Rheumatoid arthritis	N	478	652	0–2	786 Ay
20	F	69	Chronic auricular fibrillation	D	425	565	Ay	
21	F	69	Spondylarthritis	—	453	467	0–1	
22	M	70	Diabetes mellitus	A	231	603	0–2	402 Ay
23	F	71	Diabetes mellitus	—	302	382	0–1	
24	F	71	Arteriosclerotic heart dis.; Paget's dis.	D	600	605	Ay	
25	F	71	Osteoarthritis	A	715	692	0–2	
26	F	73	Old fracture of hip	N	547	880	Ay	
27	F	74	Arteriosclerotic heart dis.	D	58	116	Ay	
28	M	74	Parkinsonism	—	406	443	0–1	
29	F	75	No disease	N	606	109	0–1	
30	F	75	Arteriosclerotic heart	N	377	361	Ay	
31	F	76	Retina detachment; senility	N	488	524	Ay	
32	M	76	Diabetes mellitus	A	525	632	0–2	757 Ay
33	M	78	Obesity	—	354	330	0–1	
34	M	80	Obesity; hypertension	—	198	302	0–1	
35	F	80	Arteriosclerotic heart disease	A	34	342	Ay	
36	M	81	General arteriosclerosis	A	481	648	0–2	586 Ay
37	F	82	Old fracture of hip	N	1071	770	Ay	
38	F	83	Osteoarthritis; arteriosclerotic heart dis.	A	502	553	Ay	
39	F	85	Old fracture of hip	A	717	804	Ay	
40	M	85	General arteriosclerosis; senility	N	640	813	Ay	
41	M	86	Pulmon. emph.; arterioscler. ht. dis.	N	437	370	Ay	
42	F	88	Hemiplegia; deafness	D	401	495	Ay	
43	F	90	Healed fracture of hip; dwarfism	N	1227	1049	Ay	
44	M	90	General arteriosclerosis; senility	A	575	723	0–2	761 Ay
Mean and standard deviation					470 ± 236	553 ± 228		
Median					451	565		

* N—present in normal or increased amount; D—present in markedly decreased amount or traces; A—absent entirely; ——not determined.

† O-1: Intrinsic factor concentrate without B_{12}, Organon, Inc., in a dose equivalent to that contained in 1 U. S. P. unit of "Intrinsic Factor Preparation with Vitamin B_{12}."

‡ O-2: Same, in a dose contained in 2 U. S. P. units.

§ Ay: Intrinsic factor concentrate without vitamin B_{12}, Ayerst Laboratories, in a dose of 100 mg.

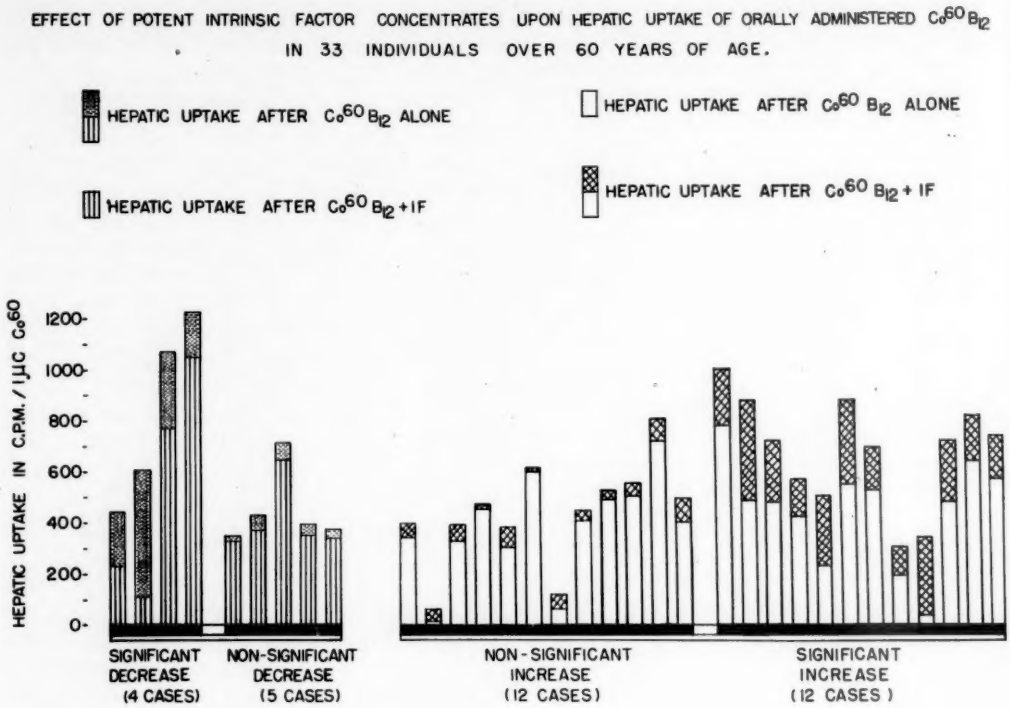
EFFECT OF POTENT INTRINSIC FACTOR CONCENTRATES UPON HEPATIC UPTAKE OF ORALLY ADMINISTERED Co60 B12
IN 33 INDIVIDUALS OVER 60 YEARS OF AGE.
HEPATIC UPTAKE AFTER Co60 B12 ALONE
HEPATIC UPTAKE AFTER Co60 B12 + IF
HEPATIC UPTAKE AFTER Co60 B12 ALONE
HEPATIC UPTAKE AFTER Co60 B12 + IF
HEPATIC UPTAKE IN C.P.M. / 1µC Co60
1200
1000
800
600
400
200
0
SIGNIFICANT DECREASE (4 CASES)
NON-SIGNIFICANT DECREASE (5 CASES)
NON-SIGNIFICANT INCREASE (12 CASES)
SIGNIFICANT INCREASE (12 CASES)

Figure 3.

in the intestine,[15] the inference is justified that there is no evidence that in advanced age the intestinal absorption of vitamin B_{12} is generally decreased.

However, data in Table I indicate that there is some tendency to a decrease in the hepatic uptake of Co^{60}-vitamin B_{12} in the age group between 50 and 80, as compared to that of young people, and that after the age of 80 this trend appears to be reversed again. Although the data are too limited in scope to allow the evaluation of the statistical significance of this trend, the statement is probably correct that in the older age group more individuals have impaired intestinal absorption of vitamin B_{12} than younger ones. The reason for this will be discussed below.

The data (Table II) also indicate that in individuals over 60 years of age the hepatic uptake of radioactive vitamin B_{12} is significantly lower in subjects with gastric hypo- or anacidity than in individuals of the same group with normal or hyperacid gastric secretion. The inference appears justified that old people with hypo- or anacidity have decreased intestinal absorption of vitamin B_{12} as compared to individuals of similar age but with normal gastric acidity.

These inferences are in apparent contrast to Schilling's findings obtained in individuals of various age groups by the use of his urinary excretion technique, on the basis of which he concluded that no difference exists between the intestinal absorption of vitamin B_{12} in individuals having normal or abolished gastric acidity.[5] It is possible that the technique of hepatic uptake measurement is able by virtue of its sensitivity to detect small differences in absorption of vitamin B_{12} between various individuals which cannot be detected by the urinary excretion technique. Halsted and associates[16] determined fecal excretion of Co^{60}-vitamin B_{12} in three patients below the age of 60 with histamine-refractory anacidity and obtained data in line with Schilling's findings. It must be remembered, however, that our data deal

TABLE IV

Effect of an Intrinsic Factor Preparation upon the Hepatic Uptake of Co^{60}-B_{12} In 30 Individuals Mainly over 60 Years of Age

Group of cases	Number of cases	Test	Hepatic uptake in counts/min/μc Co^{60} (Mean and standard deviation)	Statistical evaluation			
				Difference of means	Stnd. error of difference	T	P
Normoacidity	13	Co^{60}-B_{12} alone	570 ± 257	50	105	0.47	Non-significant
		" + I.F.	620 ± 261				
Hypoacidity or anacidity	17	Co^{60}-B_{12} alone	437 ± 227	188	84	2.24	0.05
		" + I.F.	625 ± 249				

I. F. intrinsic factor

mainly with individuals in the older age group, in whom the significance of gastric anacidity is different from that in the young subjects. Whereas in a young or middle aged population gastric anacidity frequently reflects only the isolated secretory defect of parietal cells, without impairment of secretory activity of other cellular elements of gastric glands producing pepsin, mucoprotein, or intrinsic factor, the anacidity or hypoacidity in the old age group reflects rather a comprehensive defect of gastric glands, which usually embraces all three cellular secretory glandular elements, i.e., parietal cells, peptic cells, and mucoid cells of the neck. This may explain differences in the intestinal absorption of vitamin B_{12} in the young and old patients with gastric anacidity.

It should be noted that in three individuals of the older age group with gastric hypoacidity, (Table III), the hepatic uptake was exceedingly low, ranging from 0 to 58 counts per minute per μc Co^{60}. In two of them (Nos. 15 and 27) hypoacidity was found, and the addition of intrinsic factor preparation raised the hepatic uptake only slightly (to 58 and 116 counts per minute per μc Co^{60}, respectively). In the third case (No. 35), in whom complete anacidity was found, the hepatic uptake was also almost negligible (34 counts per minute per μc Co^{60}), but the addition of intrinsic factor preparation markedly enhanced intestinal absorption, resulting in a hepatic uptake of 342 counts per minute per μc Co^{60}.

The first two cases show an absorption pattern of vitamin B_{12} in the intestine somewhat similar to that seen in sprue. The third case (No. 35) behaved like pernicious anemia, and it may represent a non-diagnosed case of pernicious anemia in remission, or perhaps a precursor of this disease. Although we have no further data to confirm these suppositions, it appears worth while to follow this lead in the future, so as to recognize and differentiate vitamin B_{12} absorption defects in the aged at an early pre-anemic stage.

The addition of a potent intrinsic factor preparation in a dose equivalent to that contained in 1 or 2 U. S. P. units of vitamin B_{12}-plus-intrinsic factor preparation *increases* hepatic uptake of radioactive vitamin B_{12} over 25 per cent in almost one-half of our cases (in 20 out of 44). In as many others no significant change was observed; in about 10 per cent of the cases a significant *decrease* of hepatic uptake was observed after addition of intrinsic factor preparations.

The increase in the hepatic uptake was observed in all age groups, but it occurred more frequently in individuals having defective gastric secretory function, as judged by decreased or abolished gastric acidity. On the other hand, the decrease in hepatic uptake following addition of intrinsic factor preparation was observed mainly in patients having normal gastric acidity. This is in line with observations indicating that sometimes an excess of intrinsic factor may cause a paradoxical decrease in vitamin B_{12} absorption.[17,18] The reasons for this effect are still not sufficiently explored, but it is probable that this phenomenon is due to excessive binding of vitamin B_{12} by inert proteins present in intrinsic factor concentrate, thereby withholding it from absorption and retaining it in the lumen of the intestine.

In a large percentage of our cases the addition of potent intrinsic factor preparation to vitamin B_{12} improved the absorption of the vitamin, in spite of the fact that these subjects neither suffered from pernicious anemia nor had undergone a total gastrectomy. The extent of this enhancing effect varies from case to case, but was very considerable (Table III) (Fig. 3).

Thus, the application of intrinsic factor preparations is no longer limited to patients with pernicious anemia or total gastrectomy,[19] but it also comprises a larger group of partial defects in vitamin B_{12} absorption. This is not particularly unexpected if one realizes that intrinsic factor is a normal product of gastric glands, and that its secretion must be *a priori* subject to similar disturbances or defects as that of other gastric glandular products, such as hydrochloric acid or pepsin, for example. The output of intrinsic factor may not necessarily be totally abolished, as in pernicious anemia, but it may fail only in part, as is the case in regard to other gastrointestinal secretory products, such as hydrochloric acid, gastric or pancreatic enzymes, or biliary salts. These partial defects in the output of intrinsic factor are apparently frequent and may be corrected by administration of intrinsic factor, in analogy to the correction by substitution therapy of other defects in the output of other gastrointestinal secretions.

SUMMARY

The measurement of the hepatic uptake of an orally administered standard dose of Co^{60}-labeled vitamin B_{12} in 60 individuals of various age groups, 18 to 90 years of age, who were normal or had some irrelevant disorder, indicates a wide range of variation in the intestinal absorption of vitamin B_{12}.

While there is no evidence of general impairment of intestinal absorption of vitamin B_{12} as a function of advancing age, more individuals in the older group have impaired intestinal absorption of vitamin B_{12} than at the younger age.

In individuals over 60 years old absorption is significantly lower in subjects with gastric hypo- or anacidity than in the individuals of the same age group with normal or hyperacid gastric secretion. It appears that the measurement of the hepatic uptake of Co^{60}-vitamin B_{12} might be helpful in the early recognition of vitamin B_{12} absorption defects of a sprue-like or pernicious anemia-like type in this group at a yet pre-anemic and asymptomatic stage.

The addition of a potent intrinsic factor preparation increases the hepatic uptake of radioactive vitamin B_{12} over 25 per cent in almost one-half of the cases in all age groups. In as many others, no significant change was observed. Especially important, however, is the increased hepatic uptake in individuals of the older age group having a decreased or abolished gastric acidity, which is usually associated with impairment of secretory output of other gastric glandular secretions.

The range of applications of intrinsic factor preparations in the correction of defective intestinal absorption of vitamin B_{12} appears to embrace not only states of frank deficiencies of vitamin B_{12} such as pernicious anemia or total gastrectomy, but also clinically latent partial defects of absorption due to decreased output of all of the secretions from gastric glands, including intrinsic factor.

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Applications of the Urinary Tracer Test (of Schilling) as an Index of Vitamin B_{12} Absorption

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THE AVAILABILITY of vitamin B_{12} labeled with Co^{60} made it possible to measure in a quantitative fashion the absorption of vitamin B_{12} from the gastrointestinal tract in man and animals. Heinle *et al.*[1] analyzed the feces of normal subjects and patients with pernicious anemia who had been fed small doses of radioactive vitamin B_{12}, and demonstrated impaired absorption in patients with pernicious anemia correctable by the administration of normal gastric juice or intrinsic factor concentrates. Their results were duplicated by others employing the same technique; however, the difficulties attendant upon the collection and preservation of specimens, which frequently had to be continued for several days, limited the usefulness of this method as a clinical test.

Glass[2] observed that vitamin B_{12} was concentrated in the liver and successfully employed scintillation counting at various points over that organ to register significant concentrations of radioactivity following the ingestion of the labeled vitamin. This method, although difficult to quantitate, demonstrates directly the uptake of the vitamin by the liver. Patients with pernicious anemia do not show any significant hepatic uptake of vitamin B_{12} by this test, unless the oral dose is accompanied by a source of intrinsic factor. Patients with sprue show either a small or no hepatic concentration of radioactivity that is not enhanced by intrinsic factor.

Schilling[3] observed that no detectable radioactivity appeared in the urine of normal subjects receiving 2 μg of vitamin B_{12}-Co^{60} by mouth. However, if the oral dose was followed in two hours by a parenteral dose of 1 mg of vitamin B_{12}, from 3 to 25 per cent of the ingested radioactive material was recoverable in the urine excreted in the next 24 hours. In contrast, patients with pernicious anemia showed virtually no excretion of the radioactive vitamin unless the oral dose was accompanied by a source of intrinsic factor. The attractive feature of Schilling's test is that the determination is performed on urine samples. This makes it possible to handle several patients at once on an ambulatory basis in the clinic.

The present authors reported[4] that when the large parenteral dose of vitamin B_{12} was given simultaneously with the oral dose of labeled vitamin B_{12}, smaller but still significant amounts of radioactivity appeared in the urine, and the difference between normal and nonabsorbing subjects was still distinct. In our hands, this modification increased the adaptability of the method for use in the outpatient department as an aid in diagnosis and research. We have now performed about 170 determinations of this kind in various patients for different purposes; this experience comprises the subject of the following report.

METHODS AND MATERIALS

The tests were performed upon inpatients and outpatients at St. Luke's Hospital. Because the original method of Schilling,[3] in

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which a parenteral dose of 1 mg of non-radioactive vitamin B_{12} is given two hours after an oral dose of 2 μg of Co^{60}-labeled vitamin B_{12}, shows slightly greater amounts recoverable in the urine, this method was used for patients in the hospital. However, to relieve outpatients from having to wait for the injection, the parenteral dose was given to them at the same time as the oral material.

Various intrinsic factor concentrates in capsule form provided by Merck & Co., Inc., Rahway, N. J., and gastric juice obtained from normal subjects, were used as sources of intrinsic factor.

The urine excreted during the next 24 hours was collected and assayed for radioactivity by scintillation counting using a 1 in. × 1 in. cylindrical Tl activated sodium iodide crystal. Labeled vitamin B_{12} with a specific activity of about 200 μc/mg was employed. The 2-μg dose gave a total count of approximately 27,000 counts/minute. Background radioactivity was determined on a casual urine specimen collected prior to the administration of radioactive vitamin B_{12}. In cases where the excretion of radioactive material was low, the original aliquot of urine was concentrated by boiling to a small volume. When more than one test was performed on a patient, a week was allowed to elapse between tests to ensure the disappearance of any radioactive vitamin B_{12} from the previously administered dose.

APPLICATIONS AND RESULTS

Assaying Intrinsic Factor Preparations

In 27 patients with pernicious anemia the test was employed to screen intrinsic factor preparations for activity prior to clinical testing. These patients showed an average excretion of 0.5 per cent (0.0–1.36) of the ingested vitamin B_{12}, and this low excretion was enhanced significantly by the simultaneous administration of intrinsic factor.

Results, compiled in Table I, show the clinical activity, dose and urinary tracer test responses of some of the preparations tested. Clinical activity is expressed qualitatively in terms of compliance with the criteria established by the U. S. P. Anti-anemia Preparations Advisory Board for the relationship between initial red blood cell count and maximum reticulocyte response. Urinary tracer (Schilling) test responses are reported with and without intrinsic factor in terms of "Per Cent of Dose Excreted." The net responses should be a measure of potency and, in the ideal case, would parallel clinical activity. The accuracy of the net values is ± 0.3 per cent of dose.

That a qualitative correlation exists between the two widely different methods—one measuring absorption or a fraction thereof, and the other the clinical effect of absorption—may be seen from Table I. Thus, the four clinically inactive concentrates, A through D, give but slight or negligible positive net responses, for the most part well under 1 per cent. In contrast, clinically active samples E, F, and G, and gastric juices produced net responses well over 2 per cent in almost every case. In one instance, 50 ml of gastric juice increased excretion from 0.2 per cent to 12.8 per cent, a value typical of the excretion in the average normal individual. Two less consistently clinically active preparations, H and I, however, exhibited intermediate net excretions of 1.4 per cent and 1.8 per cent of the 2-μg dose.

The fact that the active concentrates here reported appear to produce smaller net excretion than does gastric juice does not imply a restriction on the applicability of the Schilling test, but rather reflects the properties of the samples included in this particular study. Since the concentrates are of animal origin, the greater activity of the gastric juice might be due to species specificity. The variability of gastric juice specimens from different subjects is not unexpected in view of the differing capacity of normal individuals to absorb ingested vitamin B_{12}.

The reaction of a given subject to a potent preparation is fairly constant as within the three tests of preparation G in patient DeC. In general, responses of the same individual (note subjects McC., M., D. and B.) to different preparations parallel the group averages as well as clinical activity. The extensive observations with sample G reveal considerable variation between subjects. Some patients (V.Z. or N.) in whom the diagnosis of pernicious anemia was otherwise firmly established

TABLE I

Urinary Vitamin B_{12} Excretion (Per Cent of Ingested Dose of 2 μg Co^{60}-B_{12}) in Patients with Pernicious Anemia with and without Various Preparations of Intrinsic Factor (I.F.)

Sample	Clinical activity	Dose	Patient	No I.F.	With I.F.	Net excretion
A	Inactive	40 mg	Nic.	1.2	1.5	0.3
B	"	40 mg	Nic.	1.2	1.2	0.0
			Rem.	0.3	0.5	0.2
C	"	100 mg	McC.	0.3	0.9	0.6
			S.	0.0	1.0	1.0
D	"	50 mg	M.	0.0	0.6	0.6
			D.	0.0	1.2	1.2
E	Suboptimal	40 mg	B.	0.0	1.6	1.6
			A.	0.0	2.4	2.4
			D.	1.4	3.7	2.3
F	Active	60 mg	M.	0.0	2.4	2.4
G	Active	20 mg	Me.	1.0	3.1	2.1
		40 mg	McC.	0.3	4.0	3.7
		"	C.	0.0	3.5	3.5
		"	DeC.	0.0	4.3	4.3
		"	DeC.	0.0	2.7	2.7
		"	DeC.	0.0	4.2	4.2
		"	M.	0.0	3.5	3.5
		"	N.	0.0	1.7	1.7
		"	D.	0.0	3.2	3.2
		"	Ha.	0.2	3.8	3.6
		"	Eg.	1.0	4.5	3.5
		"	Ho.	1.4	9.5	8.1
		"	B.	0.2	7.1	6.9
		"	Me.	1.0	6.2	5.2
		"	Tur.	0.6	4.2	3.6
		"	Lyn.	0.2	2.9	2.7
		"	G.	0.1	3.2	3.1
		80 mg	C.Z.	0.2	3.4	3.1
		"	V.Z.	0.2	1.8	1.6
H	Active	40 mg	K.	0.4	1.7	1.3
			Me.	1.0	2.5	1.5
I	Active	50 mg	Me.	1.0	2.8	1.8
Gastric Juice	Normal A	42 ml	DeC.	0.0	5.2	5.2
" "	" B	50 ml	B.	0.2	12.8	12.6
" "	" "	50 ml	Me.	1.0	10.2	9.2
" "	" C	62 ml	K.	0.4	4.1	3.7
" "	Sprue	50 ml	McC.	0.3	3.3	3.0
Folic Acid		5.0 mg	C.	0.0	0.3	0.3
		5.0 mg	DeC.	0.0	0.4	0.4
		6.3 mg	D.	0.4	0.7	0.3

showed very low excretion with this preparation of known potency. The use of a smaller dose of radioactive vitamin B_{12} would enhance the percentage difference between tests with and without intrinsic factor, assuming that the same absolute amount of vitamin B_{12} were absorbed. It might prove necessary to detect such patients by screening with gastric juice or some intrinsic factor preparation of greater potency. On the other hand, these low excretion values may reflect increased storage in the liver rather than deficient absorption, or a more rapid passage of the vitamin through the upper part of the small intestine.

In general, preparations which did not cause an excretion of 2 per cent or more of the ingested dose produced insignificant or suboptimal clinical responses when fed to patients with pernicious anemia in relapse. By adopting a value of 2.5 per cent one would probably ex-

clude all preparations of doubtful potency.

Because it had been claimed by some that folic acid enhanced the absorption of vitamin B_{12} from the intestine, 5–6.25 μg of folic acid were administered together with the radioactive vitamin B_{12} in three patients. There was no significant increase in the amount of urinary radioactivity.

Differentiation of Sprue and Pernicious Anemia

Tests were performed on 13 patients with a clinical diagnosis of sprue which was made when megaloblastic anemia, glossitis, steatorrhea, and free hydrochloric acid in the gastric juice were present. All of the patients were in hematologic and clinical remission at the time of the test. A low level of excretion uninfluenced by intrinsic factor was observed in eight of the group. For these patients the average Co^{60} vitamin B_{12} excretion percentage was 1.1 per cent without intrinsic factor, and 0.4 per cent with intrinsic factor. In the remaining five the excretion ranged from 7.2 to 9.1 per cent without, and 3.6 to 7.8 per cent with intrinsic factor (Group A, Table II).

In three patients with sprue the Schilling urinary tracer test was performed with 10 mg of folic acid. No enhancement of vitamin B_{12} absorption was demonstrated.

Miscellaneous Diagnostic Problems

Seven patients on whom a diagnosis of pernicious anemia had been made because of megaloblastic anemia, histamine-refractory achlorhydria, and absence of conspicuous symptoms of steatorrhea, were found to have an average Co^{60} vitamin B_{12} excretion of 0.7 per cent with and without intrinsic factor. Five of these patients were from Puerto Rico. Fifty ml of gastric juice from one of these patients was found to increase the excretion of Co^{60}-B_{12} from 0.3 to 3.5 per cent in a patient with known pernicious anemia (Group E, Table II).

Three patients over 80 years of age with a low-grade macrocytic anemia and histamine-fast achlorhydria were found to have Schilling test values of 3.7, 1.7, and 1.69 per cent without, and 3.1, 1.3, and 1.68 per cent with intrinsic factor, respectively (Group C, Table II). This was considered to be poor vitamin B_{12} absorption due to an unknown cause.

Two patients were tested who had crippling combined system disease and mild macrocytic anemia, and small amounts of free hydrochloric acid in their gastric juice. They had been considered to have pernicious anemia despite the latter finding, because their anemia and neurologic symptoms appeared to respond clinically to injections of vitamin B_{12}. Their Schilling test results were, respectively, 4.5 and 9.8 per cent without, and 5.4 and 12.9 per cent with, intrinsic factor (Group D, Table II). While these patients may have had vitamin B_{12} deficiency, it was certainly not due to failure to absorb vitamin B_{12} because of lack of intrinsic factor.

In two children with pernicious anemia in whom the diagnosis had been established laboriously, by clinical testing,[5] despite the presence of free hydrochloric acid in their gastric juice, the diagnosis was easily reconfirmed by use of the Schilling test using an intrinsic factor concentrate of known activity and comparing the results with those obtained without intrinsic factor (Group E, Table II).

Every hematology clinic inherits some well patients who have had a diagnosis of pernicious anemia made on clinical grounds in the past, and who got well on liver or vitamin B_{12} therapy which they have continued to take. Prior to the advent of radioactive vitamin B_{12} it was impossible to determine whether or not such individuals really had pernicious anemia, and since no one cared to risk a neurologic relapse, they were continued on therapy, sometimes for years. By determining the radioactive vitamin B_{12} absorption it is now possible to make a more accurate diagnosis in these patients and, in some instances, to relieve them of the necessity of continuing to take injections. Table II shows the Schilling urinary tracer tests on two such patients (D and B) (Group F) for whom it was concluded that the diagnosis of pernicious anemia was probably incorrect, since their vitamin B_{12} absorption fell within the low normal range, without the addition of intrinsic factor.

Urinary tracer tests were also performed on four patients with polycythemia vera, one with

TABLE II

Urinary Vitamin B_{12} Excretion (Per Cent of Ingested Dose of 2 μg Co^{60} B_{12}) in Patients With Sprue and Miscellaneous Conditions

Group	Patient	No I.F.*	With I.F.*	Folic acid
A. Sprue	Go.	0.4	0.0	
	Ge.	—[a]	0.0	
	J.	1.4	1.6	1.2
	F.	—	0.5	
	C.	1.4	0.8	
	R.	—	0.2	
	O.	—	0.0	
	E.	1.0	0.7	
	S.	—	3.6	
	A.	—	7.8	
	B.	9.1	6.2	
	M.	7.9	5.5	
	Be.	7.2	—	
B. Diagnosed pernicious anemia (Megaloblastic anemia, no free HCl)	Br.	—	0.0	
	Ber.	2.1	0.9	3.8
(First 5 from tropics, may be sprue)	Fr.	0.3	0.6	
			1.7	
	T.	0.3	0.7	
	Ma.	1.0	0.9	0.7
	J.	0.3	0.4	
	L.	—	1.0	
			1.5	
C. Patients over 80 with no free HCl, mod. macrocytic anemia	Pr.	3.7	3.1	
	Fl.	1.7	1.7	
	D.	1.7	1.3	
D. Combined System Disease with free HCl	Gei.	9.8	12.9	
		7.7		
	Ger.	4.5	5.4	
E. Juvenile Pernicious Anemia (Free HCl)	C.Z.	0.2	3.1	
	V.Z.	0.2	1.2	
F. Diagnosed pernicious anemia in the past, no longer anemic	D.	6.6	9.9	
	B.	6.3	8.3	
G. Polycythemia Vera	Cr.	25.2	29.5	
	Ch.	17.5		
	Bl.	7.2		
	V.	7.8		
	E.	1.4	2.7	
		0.5		
Secondary Polycythemia	M.	16.9		
Acute Leukemia	K.	3.9		

[a] Some patients who exhibited no absorption with intrinsic factor were not tested without intrinsic factor.

* I.F.—intrinsic factor.

secondary polycythemia, and a patient with acute leukemia. The values obtained were 25.2, 17.5, and 7.2 per cent in three of the polycythemia vera patients and 16.9 per cent in the patient with secondary polycythemia. The patient with acute leukemia and another with polycythemia vera with a leukemic blood picture showed low excretion values of 3.9 and 0.2 per cent, respectively. (Group G Table II). Two patients with severe hepatic cirrhosis showed excretions of 10.1 and 20.9 per cent, respectively.

DISCUSSION

The relative simplicity of the Schilling urinary excretion tracer test makes it a practical

tool for the screening of patients on an ambulatory basis. The test provides useful information as to whether or not the patient is absorbing vitamin B_{12} from the intestinal tract. As yet we have available no figures comparing results in the same patient fed Co^{60}-labeled vitamin B_{12} obtained with the methods of stool assay, hepatic uptake measurement, and urinary excretion. Thus, we have no way of knowing how much of the total amount absorbed from the intestine is excreted in the urine, and the test is therefore only semi-quantitative. However, the results of repeated tests in the same individual are reasonably consistent under the same circumstances, and do permit the evaluation of the presence or absence of intrinsic factor, its role in a given case of poor absorption, and even the relative activity of different intrinsic factor preparations.

Since the Anti-anemia Preparations Advisory Board of the U. S. P. still requires that antipernicious anemia products be clinically tested in three patients with pernicious anemia in relapse, and such patients are becoming increasingly difficult to find, it is exceedingly useful to be able to screen intrinsic factor preparations for activity, and to reserve the clinical testing for those preparations of proven worth. Unfortunately, the conditions established by the Board for clinical testing permit a dose of vitamin B_{12} (15 μg) that will produce partial or even complete remission by itself in some patients with pernicious anemia. It appears inevitable that some responses actually due to the vitamin B_{12} will be attributed instead to intrinsic factor preparations which are, in fact, of inferior potency. Some "clinically active" preparations (H and I, Table I) exhibited inferior excretion responses. If one were to set a minimum net value of 2.5 per cent as a criterion for potency, nearly all preparations of questionable activity would be excluded.

The test has also served to throw light on some of the factors influencing the absorption of vitamin B_{12}. Like others,[6,7] we have found that unless there is a deficiency of intrinsic factor to begin with, additional intrinsic factor does not increase the amounts of vitamin B_{12} excreted in the urine (and presumably absorbed). In particular, the data from patients with sprue indicate that some factor *other* than intrinsic factor is essential for proper vitamin B_{12} absorption, a point which was proved both indirectly by impaired vitamin B_{12} absorption by such patients, and directly by the demonstration of intrinsic factor activity in the gastric juice of one of these patients. Glass has suggested[6] that there is a receptor protein in the mucosal cell of the intestine which accepts and binds vitamin B_{12} in a manner analogous to the iron-apoferritin system described by Granick.[8] Evidence for such a protein-B_{12} complex has been provided by Wijmenga.[9] A defect of this sort may be responsible for the failure of vitamin B_{12} absorption in sprue.

Sprue is a syndrome of malabsorption frequently accompanied by a severe megaloblastic anemia. This anemia responds to folic acid given orally or parenterally, and to parenterally administered vitamin B_{12}. However, the latter substance is usually ineffective when given by mouth to patients with sprue. Radioactive vitamin B_{12} absorption studies indicate why this should be so. In the group of patients with low urinary excretion values, insignificant amounts of oral vitamin B_{12} are absorbed, and in the group with normal urinary excretion values, there is probably no deficiency of vitamin B_{12}. The occasional patient with sprue who fails to respond to parenteral vitamin B_{12} probably falls in the latter group; such patients usually respond well to folic acid.

Many regard sprue as a state of folic acid deficiency, sometimes primary and sometimes secondary to the steatorrhea. Our studies indicate that although in sprue the patient may well have a folic acid deficiency, there is a basic abnormality in the absorption of vitamin B_{12} in many of these patients which is not due to a generalized malabsorption syndrome. This absorptive defect is present even in the absence of other manifestations of malabsorption, such as steatorrhea and poor glucose absorption, and is not influenced by either intrinsic factor or folic acid. In general, the Schilling test excretion values appeared to be slightly higher in the patients with sprue than in the patients with pernicious anemia, suggesting a slightly higher absorption of vitamin

B_{12}. It may be that the infrequency of combined system disease in patients with sprue is due to the fact that they absorb a little more vitamin B_{12} than do the Addisonian anemia patients. However, that neurologic manifestations do occur in sprue is attested to,[10] and several of our patients did show no urinary excretion of vitamin B_{12} whatsoever, by the tracer test. It is therefore our opinion that the treatment of choice for patients with sprue is parenteral vitamin B_{12}, to which oral folic acid may be added as a supplement.

The patients with sprue-like absorption patterns, who had been diagnosed clinically as having pernicious anemia because of histamine-refractory achlorhydria, are difficult to classify. Because most of them (five of seven) were from the Caribbean region, it was tempting to call this disorder "sprue with achlorhydria." Similar patterns were observed in three additional patients over the age of 80 who had no history suggestive of sprue, and whose low-grade macrocytic anemia had responded erratically to parenteral liver extract and vitamin B_{12}.

The existence of low serum vitamin B_{12} levels in the aged has been reported by Chow[11] and that the aged sometimes also have poor vitamin B_{12} absorption not influenced by intrinsic factor was demonstrated by Glass and others.[12] These elderly patients certainly do not have pernicious anemia, if one reserves that diagnosis for patients whose poor absorption of vitamin B_{12} is demonstrably corrected by intrinsic factor. Unless they had a history of some clinical manifestation of sprue they should not be thus designated either. At the present state of our knowledge it is best to consider such subjects as examples of defective absorption of vitamin B_{12} due to an unknown cause.

The diagnostic usefulness of the Schilling urinary excretion test is obvious in establishing whether or not a patient has pernicious anemia and will benefit by injections of vitamin B_{12}. There are undoubtedly many people now receiving such injections who really do not need them.

SUMMARY

Twenty-seven patients with pernicious anemia showed an average urinary excretion of 0.5 per cent of an ingested dose of 2 μg of Co^{60}-labeled vitamin B_{12} when tested by the method of Schilling. The ingestion of normal gastric juice increased this to values ranging from 4 to 12 per cent. The intrinsic factor concentrates studied increased the excretion of radioactivity to values ranging up to 7.1 per cent. A net excretion of 2.5 per cent or greater (as measured by the Schilling test) is a good criterion for an expected optimal clinical effect of the intrinsic factor preparation used in patients with pernicious anemia in relapse.

Nine of 13 patients with sprue showed an average excretion of 1.1 per cent which was not enhanced by intrinsic factor. This is interpreted as evidence that in some patients there is a deficient absorption of vitamin B_{12} from some cause other than intrinsic factor deficiency.

The urinary tracer test was useful in various clinical states to prove or disprove the diagnosis of pernicious anemia. The existence of a group of patients with megaloblastic anemia due to deficient B_{12} absorption, not caused by lack of intrinsic factor and without symptoms of sprue was demonstrated.

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The Modern Scapegoats

"People hate scientists. There is no use in beating about the bush here. The scientist is in danger of becoming the scapegoat for the helplessness which the public feels. And if an immense revulsion of public feeling does lead to the destruction of the scientific tradition, then the world may again enter a dark age as it did after the Goths destroyed Rome. It is not impossible that the whole mechanical and intellectual society which we know could be abolished by a great wave of fanaticism.

* * *

"What the lay public does when it hates the scientist is what it does also when it hates policemen and ministers of state and all symbols of authority. It tries to shift the responsibility for decisions from its own shoulders to the shoulders of other people. 'They have done this,' it says. And 'They' is always the law, the government—or in this case, the scientist."

—J. Bronowski. *Bull. Atomic Scientists* 12: 10, 1956.

The Honour of Abuse

"...As to the Abuses I meet with...you must know I number them among my Honours. One cannot behave so as to obtain the Esteem of the Wise and Good, without drawing on one's self at the same time the Envy and Malice of the Foolish and Wicked, and the latter is Testimony of the former. The best Men have always had their Share of this Treatment, and the more of it is in proportion to their different and greater degrees of Merit. A Man has therefore some Reason to be asham'd of of himself when he meets with none of it."

—Benjamin Franklin. London, March 2, 1767.

The Urinary Excretion Test for Absorption of Vitamin B_{12}

I. REPRODUCIBILITY OF RESULTS AND AGEWISE VARIATION

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SEVERAL radioactive tracer methods have been proposed for the determination of the extent of vitamin B_{12} absorption by humans. These methods employ oral test doses of 0.5–2 μg of vitamin B_{12} labeled with radioactive cobalt.[1–3] Absorption may be estimated from radiometric measurements of feces,[4] scintillation counting of liver projections,[5] or determination of urinary radioactivity estimated after injection of a massive dose of non-radioactive vitamin B_{12}.[6]

The urinary excretion test[6] is useful for the study of the effect of intrinsic factor on the absorption of vitamin B_{12} by patients with pernicious anemia; the application of this test to those without an extreme derangement in absorption mechanism is hampered by the lack of information on the reproducibility of the results obtained in clinically healthy subjects. To make a systematic study, a group of young volunteers from a single penal institution was used. These subjects were all males between 19 and 30 years of age. They had a similar dietary history and all had lived in the same environment for at least three years. These uniform conditions, together with the careful supervision of urine collections, are desirable and, indeed, essential, in quantitative studies, and have rarely been presented in previous reports.

In addition, older subjects were drawn from three homes for the aged, each providing medical supervision. All subjects were ambulatory and were apparently in good health and free from acute infectious diseases and metabolic disturbances. Twenty-four hour nursing service was provided specifically to assure a complete urine collection. Some of these persons were subjected to repeated tests in order to study variations in individual responses. A total of 40 normal adult men, ranging in age from 19 to 85 years, were studied. These experiments provide information concerning the variation in absorption of vitamin B_{12} between individuals of various age groups living under somewhat comparable conditions.

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EXPERIMENTAL

Test Procedure

The procedure employed is essentially that outlined by Schilling.[6] Two hours after the oral administration of 2 μg of labeled vitamin B_{12} to the test subjects, one milligram (1000 μg) of crystalline vitamin B_{12} in physiologic saline solution was injected intramuscularly, and urine specimens were collected for a period of 24 hours. In all but three cases, 24-hour collections were made in two successive periods of (1st period) 0–8 or 0–12 hours and (2nd period) 8–24 or 12–24 hours. In nine cases, a second 24-hour collection was made. An aliquot of each collection, equivalent to one-quarter of the total urine collected, was evaporated to 50 ml in a beaker containing 50 μg of vitamin B_{12} as carrier, and the radioactivity of the concentrate was measured by scintillation counting[7] using a thallium-activated sodium iodide crystal. The radioactivity measured in this manner was corrected to the total volume of urine, and computed as per cent of oral dose excreted. The activity

of the 2-μg dose measured in this manner was about 25,000 counts per minute. In 31 cases an aliquot of the original urine was assayed microbiologically for total vitamin B_{12} content using *L. leichmannii*.[8]

Intrinsic Factor Used

In view of the known efficacy of intrinsic factor concentrates in promoting the absorption of orally administered vitamin B_{12} by patients with pernicious anemia, eight individuals were tested with two oral intrinsic factor concentrates to explore the effect of such preparations in normal persons. One preparation (I) was known to be clinically active at 40 mg per day and the second preparation (II) was proved active at 2 mg or less per day. An oral dose of 40 mg of preparation I or 2 mg of preparation II was administered with the tracer vitamin.

TABLE I

Excretion of Vitamin B_{12} by Normal Subjects of Diffierent Ages

Age (yr)		No. of subjects	% of Radioactivity excreted	Average % of 24-hr total in first period urine (0–8 or 0–12 hr)	
Range	Mean		Mean and Range	Radioactive	Microbiol.
19–25	22.3	12	10.2 ± 2.8* (4.5–13.8)	36	89
26–40	30	5[a]	13.0 ± 2.8 (8.4–17.7)	31	77
41–55	49.5	6	13.4 ± 4.7 (5.3–19.7)	29	58
56–70	60.4	5[b]	8.2 ± 3.7 (2.3–13.3)	9	41
71–85	77.1	12	12.3 ± 6.2 (1.8–25.6)	25	63

* Values following ± symbol are standard errors of the means.

[a] In three of these cases, a single 24-hour collection was made. Microbiologic assays were performed only for the other two subjects. Ratios apply only to these two subjects.

[b] The apparent lower excretion rate shown by the 56–70 year groups is due to the shorter duration of the first collection period, i.e., 8 hours compared to 10 to 12 hours.

RESULTS

Age Group Averages

Responses obtained by the Schilling urinary excretion test are reported in Table I. The results are presented by age groupings. The radioactivity and microbiologic activities excreted in the second 24-hour interval were usually well below 1 per cent of the oral dose and are not included in these figures. Urinary excretion of the injected dose of 1 mg of crystalline vitamin B_{12} was essentially complete in 24 hours, subsequent elimination being inappreciable. In the last two columns of Table I are listed group average percentages of the total 24-hour output which appeared in the urine during the first collection period for both radioactivity and microbiological measurement.

It should be pointed out (Table I) that on the average the bulk of the *orally* administered radioactive vitamin which is flushed out is eliminated in the second collection of the 24-hour period, whereas microbiologic assays indicate a preponderant excretion of *injected* vitamin B_{12} during the first collection period (0–12 hours). Thus, group averages ranging between 9 and 36 per cent of the total radioactivity from the orally administered labeled vitamin B_{12} excreted in 24 hours appear in the first period urines, while elimination of the unlabeled vitamin B_{12} ranged from 41 to 89 per cent in the same period after the intramuscularly administered dose. In only 4 of the 40 cases reported in Table I did the radioactivity of the first period urine exceed that in the second collection, and then by very little; even in these cases total vitamin excretion was more rapid than the excretion of radioactivity. Similarly, four cases were noted in which the quantity of unlabeled vitamin B_{12} in the initial urine was less than that in the second collection; but these subjects still exhibited a relatively much greater initial elimination of nonradioactive vitamin than of the radioactive form. The elimination of the injected vitamin B_{12} is practically complete (average 91 per cent) in 24 hours, compared to the limited excretion (average 11.4 per cent) of the oral dose

of the radioactive vitamin. The rapid and quantitative elimination of large doses of parenteral vitamin B_{12} has been reported elsewhere.[9,10]

Reproducibility

To study the reproducibility of results on repeated tests with the same individuals, a group of eight subjects, age 20 to 26, were given 2 μg of radioactive vitamin B_{12} by mouth in two successive studies conducted two months apart. The results of repeated tests are shown in Table II. The maximum difference between the first and second tests was no more than 2 per cent of the administered dose and the variation with time was random. By and large, the duplicate tests were reproducible to within 10 to 15 per cent of the initial value.

TABLE II

Repeated Tests of Urinary Excretion of Radioactive Vitamin B_{12} in Young Subjects

Subject's age	First trial	Second trial	Difference (First trial − second)
yr	%	%	%
20	8.9	9.4	−0.5
21	12.0	10.2	+1.8
21	7.7	8.6	−0.9
24	10.2	10.6	−0.4
24	7.5	8.2	−0.7
26	11.8	9.8	+2.0
26	8.6	8.2	+0.4
26	11.1	12.3	−1.2

A group of eight subjects, aged 34 to 85 years, were also given 2 μg of radioactive vitamin B_{12} orally in successive tests. Furthermore, the effect of intrinsic factor concentrate was examined in ten experiments. Results of successive tests, again expressed as per cent of dose excreted, are shown in Table III. These values were not included in the averages shown in Table I.

The results in Table III demonstrate that the largest difference in the per cent of excretion of radioactivity in the urine of the test individuals in the two successive trials without intrinsic factor was 6 per cent of the administered dose (subject: age, 85), although in a majority of cases, the mean absolute difference was only 2.3 per cent.

Of the seven subjects who were tested some two weeks later with an intrinsic factor preparation at the time of the tracer study, three responded with an increased output, whereas four had a decreased excretion. A second test with the more potent preparation (II) was also equivocal. Such variations are not unexpected, since our "older" individuals cannot be considered as homogeneous subjects.

TABLE III

Repeated Tests with and without Intrinsic Factor Concentrate

Per cent of orally administered radioactivity in urine

Subject's age	First trial		Second trial	
	No IF (3/25/54)	Plus IF (4/9/54)	No IF (5/18/54)	Plus IF (7/13/54)
yr				
34	13.1	16.9 (I)*	—	—
53	14.3	12.3 (I)	14.7	—
56	8.8	3.1 (I)	9.1	—
61	2.3	4.8 (I)	3.8	9.1 (I)
63	6.6	4.4 (I)	8.7	5.9 (I)
77	14.6	—	9.4	—
83	1.8	9.2 (II)*	2.4	6.2 (II)
85	13.2	9.2 (II)	19.2	—

* I or II in parentheses denote the intrinsic factor (IF) preparations used (see text) and all figures are expressed as per cent of the radioactive vitamin administered orally.

It is noteworthy that the two subjects (ages 61 and 83) who exhibited low initial responses excreted more radioactive vitamin B_{12} in the urine after the administration of intrinsic factor, whereas the remaining subjects with essentially normal initial excretion received no benefit from the preparation. The lack of effect on the latter group is an indication of the probable normalcy of absorption, which apparently cannot be enhanced by exogenous intrinsic factor preparation.

DISCUSSION

Variations among individuals on repeated tests may occur under certain test conditions. For example, if the injection of the "flushing" dose of 1 mg of vitamin B_{12} is carried out at $^1/_2$ or 3 hours instead of 2 hours after the oral dose, the urinary excretion may be decreased by 50 per cent. Furthermore, the amount of radioactivity in the urine cannot be increased by more frequent "flushing" with a larger total

dose of injected vitamin B_{12}. Thus, injection of a total of 4 mg of crystalline vitamin B_{12} every two hours resulted in excretion of 10.8 ± 2.2 per cent (10 subjects) of radioactivity in 24 hours. The same amount was excreted after a single injection of 1 mg after two hours.

Whether this test is an important index of vitamin B_{12} absorption remains to be answered by further experiments. However, available data demonstrate that the radioactivity "flushed out" in the urine represents only a fraction of the oral vitamin B_{12} actually absorbed by a subject. It has been shown that absorbed vitamin B_{12} is retained in large part by rats[11] or man[12] for a long period of time, at least 6 months. Glass, Boyd, and Stephanson[12] found by means of the hepatic uptake method that about 40 per cent of a 2 μg oral dose is absorbed. This is in substantial agreement with the value of 50 per cent reported by Swenseid, Gasster, and Halsted[13] using the fecal excretion method. On this basis our overall average value of 11.4 per cent flushed out in the urine corresponds to about one-fourth of the total absorbed vitamin. Despite the fact that absorbed vitamin B_{12} circulates through the blood stream and is retained in certain organs, the amount excreted still represents a large enough fraction to constitute a significant measure of the total amount absorbed.

The data in Table I do not indicate an abnormal variation of the group average response with age; nor do individual responses among the persons examined fluctuate markedly. Such responses may be considered an index of the absorptive capacity of the test subjects. If we assume that these responses are a measure of absorption, we are justified in using urinary excretion in comparing absorption of vitamin B_{12} as a function of age. Such an assumption does not recognize the possible differences in renal function and in tissue retention for vitamin B_{12} between subjects of different ages. Examination of the data in Table I fails to reveal any significant difference in response between the several age groups.*

* Since this paper was written a report by Schilling (*Am. J. Clin. Nutrition* 3: 45, 1955) was published in which the same conclusion was made.

It is of interest to point out that there were some isolated cases of extremely low absorption. One subject, aged 61 years, gave a response of only 2.3 per cent, while another subject, aged 81, excreted only 1.8 per cent of the oral radioactive dose. Besides these unusually low responses, one case each of 5 per cent excretion was noted in the 19–25 and 41–55 age groups. It is conceivable that these individuals suffered from some incipient dysfunction which had not yet fully developed, and that the low urinary excretions observed need not reflect poor absorption due to aging *per se*.

In a previous communication[14] it was reported that, following the oral administration of a test dose of 1000 μg of vitamin B_{12}, a positive response (i.e., a rise in vitamin B_{12} level in plasma) is less frequent in subjects 65 years or older than in persons between 20 and 35 years old. While these findings might be construed as indicating a defective absorption mechanism in the aged, it must be recognized that the dose of 1000 μg was far in excess of physiologic requirements. The current study, which reduces the test dose to a more physiologic level, appears on the surface to contradict the observations based upon the plasma assays. However, the discrepancy probably results from the 500-fold difference in oral dose. It is plausible that the older individuals may possess enough intrinsic factor to aid the absorption of 2, but not 1000, micrograms.

SUMMARY

A urinary excretion test using radioactive vitamin B_{12} was applied to a group of young male adults with similar dietary histories and living conditions and under conditions which minimize errors in urine collections, as well as to a group of ambulatory elderly individuals. The variation of the results obtained from this group of normal subjects and the reproducibility of the tests performed on the same individuals are presented. The results failed to reveal a significant variation of absorption with age. Of interest is the small incidence of low absorption in the aged group. It is not certain to what extent these measurements represent true absorption, since urinary ex-

cretion of orally administered vitamin B_{12} may be influenced by tissue retention and kidney function. The simultaneous administration of intrinsic factor concentrates to subjects without pernicious anemia does not appear to enhance the absorption of vitamin B_{12}. Variations observed were not greater than the normal variation exhibited by a given subject.

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The Urinary Excretion Test for Absorption of Vitamin B_{12}

II. EFFECT OF CRUDE AND PURIFIED INTRINSIC FACTOR PREPARATION

By BACON F. CHOW, PH.D., WILLIAM L. WILLIAMS, PH.D., KUNIO OKUDA, M.D., AND RALPH GRASBECK, M.D.

CASTLE postulated the presence in normal gastric juice of a substance which he called intrinsic factor. The biological activity of this substance is shown by the response of subjects with pernicious anemia to its co-administration with oral vitamin B_{12}. While this hypothesis is now well established, the effect of this substance on the absorption of the vitamin by normal individuals has not been studied in detail. Rosenblum[1] and Chow[2] and their associates demonstrated that certain intrinsic factor preparations of hog origin may even interfere with the absorption of orally administered radioactive (Co^{60}) vitamin B_{12} by rats, in that the addition of this agent would actually increase the fecal excretion of radioactivity. These results were interpreted to indicate a possible inhibition of absorption of vitamin B_{12} given by mouth due to the administration of crude concentrates to rats. Swendseid[3] reported a similar adverse effect of intrinsic factor concentrates in totally gastrectomized subjects. No systematic study has yet shown whether or not intrinsic factor aids vitamin B_{12} absorption by clinically healthy humans.

We have recently reported on the variability of the urinary excretion test[4,5] among normal persons and it was, therefore, of interest to apply this procedure to such a study. In this communication we wish to present the results of feeding various intrinsic factor preparations to healthy, young individuals, as measured by the urinary excretion of the radioactivity following the administration of labeled vitamin B_{12}. Our results show that the inhibitory effect of intrinsic factor material shows itself when certain crude, but not purified, preparations are used.

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EXPERIMENTAL

Test Procedure

The procedure employed is essentially that described in our previous communication.[5] Two hours after the oral administration of the desired amount (2 or 50 μg) of cobalt^{60}-labeled vitamin B_{12}[6] with or without the intrinsic factor preparation being studied, 1 milligram of crystalline vitamin B_{12} in physiologic saline solution was injected intramuscularly. Urine specimens were collected for a period of 24 or, in some instances, 48 hours, under carefully supervised conditions. An aliquot of each collection, equivalent to one-quarter or one-half of the total volume collected, was evaporated to 50 ml in a beaker containing 50 μg of unlabeled vitamin B_{12} as carrier. The concentration was measured by scintillation counting, using a thallium-activated sodium iodide crystal. The radioactivity measured in this fashion was corrected to the original volume of urine and computed as per cent of oral dose excreted. The activity of one microgram was equivalent to about 25,000 to 30,000 counts per minute. Although it is not certain to what extent the urinary excretion test represents a measure of true absorption of vitamin B_{12}, this

method is convenient and useful both for qualitative determination of intrinsic factor preparations and effective comparisons of materials.

Choice of Subjects

All subjects used in the present study were residents of a state penal institution for at least three years and had comparable dietary histories. They were clinically healthy and free from acute infectious diseases or metabolic disturbances at the time of the tests. During the first twelve hours of the urine collection periods they were required to stay in one room and were denied toilet facilities without the consent of the supervisor. They were then allowed to sleep in their dormitories. On the following morning they were again requested to stay in one room in order to complete the 24- or 48-hour collections. The comparability of the nutritional state of the test subjects, which might be inferred from the controlled dietary of the institution, and the satisfactory condition for the urine collection provide a very desirable and, indeed, necessary prerequisite for a study of normal metabolism. To insure complete urinary collections, some specimens were analyzed for creatine nitrogen and for microbial activity of vitamin B_{12}. Usually at least 90 per cent of the injected vitamin B_{12} or 1.8 g creatine nitrogen were found in the urine.

Intrinsic Factor Preparation

Two batches of intrinsic factor preparations obtained by the extraction of hog stomach and intestines with ammonium sulfate or alcohol were used. Their intrinsic factor potencies, as established in patients with pernicious anemia in relapse, were found to be such that about 25 and 50 milligrams, together with 15 micrograms of vitamin B_{12}, comprised one U.S.P. unit, for preparations *A* and *B*, respectively. A highly purified preparation (C), less than 1 mg of which together with 15 μg of crystalline vitamin B_{12} by mouth gave a satisfactory response in suitable test subjects, was also used.

RESULTS AND DISCUSSION

The results of five separate trials (a–e) in which 2.0 μg of radioactive vitamin B_{12} were given to 41 young male individuals, and which were carried out over a period of seven months, are collected in Table I. Each trial reported in this communication is designated by a letter. Similar letters indicate that the trials were carried out concurrently. At least three batches of radioactive vitamin B_{12} were used. A striking finding is that with 2-μg doses of radioactive vitamin B_{12} the mean urinary excretion of radioactivity varied only from 204 to 240 millimicrograms within 24 hours. Fifty μg of radioactive vitamin B_{12}* were given orally to another 26 subjects in four trials (b, d, e, f). The urinary output of radioactivity increased to a range of means of 442–491 millimicrograms in three separate trials; in the fourth (study b), an unusually large variation was encountered because of the low specific activity of the labeled vitamin B_{12} fed, and there was insufficient activity in the urine for accurate measurement.

TABLE I

Urinary Excretion of Radioactive Vitamin B_{12} after Oral Administration

Amount of B_{12} given (μg)	Study	Labeled vitamin B_{12} in urine (24 hr) mμg ± S.E. of the mean
2	a (12)	204 ± 18
	b (7)	244 ± 38
	c (8)	240 ± 44
	d (8)	216 ± 24
	e (6)	210 ± 20
50	b (6)	502 ± 52
	d (7)	454 ± 35
	e (7)	442 ± 30
	f (6)	491 ± 32

( ) Denotes the number of subjects used.

In Table II are tabulated the results of three trials involving the use of 59 different individuals. In each trial (d, e, f), a separate control group was used. Fifty-microgram doses of radioactive vitamin B_{12} were fed orally with or without the simultaneous administration of one or another of the intrinsic factor preparations. In trial (d) the administration of 50 μg of vitamin B_{12} resulted in the urinary excretion of approximately 454 ± 35 mμg of radioactive

* Generously supplied by Dr. Rosenblum of Merck and Company, Rahway, New Jersey.

vitamin B_{12}. In the same trial the administration of 4 U.S.P. equivalents of intrinsic factor preparation *A*, together with 50 mμg of labeled vitamin B_{12} resulted in a decrease to 332 ± 46 mμg. The difference between the mean urinary excretion is statistically significant ($P < 0.05$). Similar decreases were obtained in two other trials (e and f) using the same preparation; they were likewise statistically significant.

TABLE II

Effect of Intrinsic Factor Preparations (I.F.P.) on Urinary Excretion of Orally Administered Radioactive Vitamin B_{12}*

Trials	Treatment Vit. B_{12} (μg) + I.F.P. (mg)		No. of Subjects	Radioactivity in 24-hr urine mμg ± S.E.
d	50	0	7	454 ± 35
	50	100 (A)	7	332 ± 46
e	50	0	7	442 ± 30
	50	100 (A)	6	354 ± 20
	50	200 (B)	5	365 ± 36
	50	12 (C)	6	525 ± 40
f	50	0	6	491 ± 32
	50	100 (A)	8	364 ± 26
	50	12 (C)	7	609 ± 33

* All subjects were between 22 and 33 years old.
(A), (B), (C) denote preparations of intrinsic factor concentrates used (see text).

Table II also demonstrates clearly that another crude preparation, *B*, was equally inhibitory to oral absorption, causing a decrease in urinary excretion of labeled vitamin B_{12} of from 442 to 365 mμg. To test the possibility that the decrease in radioactivity in the urine may have been due to a delay in excretion, the collections of urine in some trials were extended from 24 to 48 hours. The second 24-hour period collection failed to increase the recovered urinary radioactivity by more than 5 per cent either in the control or the experimental group (i.e., with and without intrinsic factor). Therefore, delayed excretion was not a factor. These data, taken as a whole, therefore indicate that under our test conditions, both crude intrinsic factor concentrates in some way decreased the urinary excretion of subjects receiving radioactive vitamin B_{12}.* It cannot be predicted whether such effects could be observed if the ratio of vitamin B_{12} to intrinsic factor preparations were altered. In trial (e), 50 μg of vitamin B_{12} were also given to two additional groups of 8 subjects each, together with 1 or 16 U.S.P. units equivalent of preparation *A*. Our results showed that one U.S.P. unit equivalent caused no inhibitory effect (450 ± 25 mμg), but 16 U.S.P. units equivalent decreased the urinary excretion to 225 ± 20 mμg from a control value of 442 ± 30 mμg.

A question of importance is whether the decreased urinary excretion is due to intrinsic factor *per se* or to some inhibitory substances present in the concentrate. To this end, preparation *C* derived from preparation *A* was tested. In the ultracentrifuge and by electrophoresis, it contains a major unsymmetrical peak (75%) and a minor one (25%). The administration of 12 mg of this purified substance caused a statistically significant increase in urinary excretion above that seen when labeled vitamin B_{12} was given alone, or with a more crude extract (*A*) (Table II). These data, therefore, suggest that the inhibitory effects on urinary excretion noted with crude preparations are, perhaps, associated with substances other than intrinsic factor itself; these appear to have been removed during the purification procedure used to prepare substance *C*.

Conversely, it should be pointed out that not all crude intrinsic factor preparations contain inhibitory substances. For example, it was found that the co-administration of 4 units of a preparation† (50 mg/U.S.P. unit) with 50 μg of radioactive vitamin B_{12} resulted in an increase of 24-hour urinary excretion from a mean value of 510 ± 40 mμg for the control group (8 subjects) to 660 ± 30 mμg (8 subjects). The difference is statistically significant. These data indicate then that this preparation is not inhibitory.

The amount of radioactivity excreted in the urine of our test subjects is dependent not only on the absorption but also on the tissue retention and renal function. Thus, the decrease in urinary excretion following the administration of certain intrinsic factor preparations can

* Made available to us through the courtesy of Dr. William Williams of Lederle Laboratories.

† "Neotinic" made available to us by Stuart Co., Burbank, California.

be considered only as suggestive evidence that such concentrates may inhibit absorption of orally administered vitamin B_{12}. Although we do not believe that differences in tissue retention or in renal function existed among our subjects in randomly selected groups, supporting evidence of the inhibitory effect must await the studies using other independent criteria, such as the determination of fecal excretion of orally administered radioactive vitamin B_{12} or the oral tolerance test for changes in blood vitamin B_{12} levels following prolonged oral feeding of unlabeled vitamin B_{12}.

TABLE III

Vitamin B_{12} Binding Power of Intrinsic Factor Preparations Used

Preparation	mg/unit*	Millimicrograms vitamin B_{12} bound/mg I.F.P.	
		Microbiologic method	Dialysis
Crude A	50	37.5	53.0
Crude B	200	49.0	68.0
C (Purified A)	1 or less	54.0	65.0

* Antipernicious anemia unit as defined—U.S.P.

It is also of interest to point out that the highly purified preparation *C* is still capable of binding vitamin B_{12}, as determined by our microbial test[8] or by dialysis.[9,10] The data in Table III demonstrate that the binding power of the purified material was equal to or even greater than that of the crude preparations (consisting of more than 6 electrophoretic components) when expressed on the basis of vitamin B_{12} bound per milligram of dry powder. These facts thus provide additional suggestive evidences for our hypothesis[11] that intrinsic factor binds vitamin B_{12}, although not all binding substances in the extracts of hog stomach or intestine possess intrinsic factor activity.

SUMMARY

A systematic study involving the use of 91 young, healthy volunteers demonstrated that certain crude intrinsic factor preparations decreased the urinary excretion of orally administered radioactive vitamin B_{12}. On the other hand, a highly purified preparation derived from the crude concentrate or a special preparation with no inhibitory substance increased the urinary output of radioactivity. Thus, the observed decrease in urinary excretion is not due to intrinsic factor *per se*, but to some interfering substances present in our crude concentrates. The purified preparation is still capable of binding vitamin B_{12} according to the microbial absorption procedure or by the dialysis method.

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Studies of Niacin Requirement in Man

III. COMPARATIVE EFFECTS OF DIETS CONTAINING LIME-TREATED AND UNTREATED CORN IN THE PRODUCTION OF EXPERIMENTAL PELLAGRA

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PREVIOUS studies of the role of corn diets in the production of pellagra indicated that niacin deficiency was induced less readily in subjects maintained on "wheat" diets than in subjects receiving "corn" diets of comparable niacin and tryptophan content.[1] The time required for the development of pellagra, and the severity of the deficiency, appeared to be related to the intake of niacin and tryptophan per unit of body size. However, this relationship did not seem to be the complete explanation of the apparently greater pellagragenic effect of corn- than of wheat-containing diets.

Kodicek[2] reported that corn contained "bound" niacin which was unavailable to the rat unless the corn had been subjected to alkaline hydrolysis. Laguna and Carpenter[3] found that growth depression induced in rats by feeding high corn diets was diminished by treatment of corn with lime. The presence of "bound" niacin in corn might be a partial explanation of the corn-pellagra relationship The incidence of pellagra is relatively low in some areas, such as Central America, where corn is the staple cereal but is treated with lime prior to incorporation in the diet.

The present investigation was designed to determine the effect of treatment of corn with lime on the production of experimental pellagra and to explore the possibility that the presence of "bound" niacin in corn might explain, in part, the pellagragenic effect of this cereal.[4]

EXPERIMENTAL

Six metabolic studies were carried out in four white female subjects who were essentially free of organic disease and who ranged in age from 43 to 57 years. The subjects were maintained in a metabolism ward and were ambulatory throughout the period of study. After a preliminary period* of at least three weeks during which a good varied diet was administered, the subjects were placed on corn diets low in niacin and tryptophan. In three instances, the corn was treated with lime before being incorporated in the diet; in the other three the corn was untreated. Unenriched corn, obtained in the United States and milled with the usual processes, was used in the first experiment; whole corn, grown in Guatemala, in the other five. The corn which was treated with lime was prepared in Guatemala in the manner usual in the preparation of tortillas, through the co-operation of Dr. Nevin Scrimshaw and his associates of the Institute of

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* The preliminary period was omitted in experiment 6 (subject O. B.).

Nutrition of Central America and Panama.* The untreated whole corn from Guatemala was ground and used as grits or cornmeal. The moisture content of the untreated corn and the lime-treated corn, which is termed "masa," was determined and found to be 10 per cent and 62 per cent, respectively. The two types of corn were incorporated in the diets in amounts approximately equivalent to 80 grams dry weight. The niacin content of masa, and of untreated cornmeal and grits, was determined microbiologically[5] and found to average 16 micrograms and 17.4 micrograms per gram, respectively, on a dry weight basis.

TABLE I

Corn Diet Low in Niacin and Tryptophan

Breakfast	g	*Supper*	g
Orange or grapefruit juice	200	Beets or carrots	100†
Corn grits or	45*	Corn bread or	75‡
Masa mush	100*	Masa mush	75‡
White bread	30	Oleomargarine	10
Oleomargarine	10	Grape juice	100
Sugar	10	Sugar	30
		Gelatin	20
		Pears or pineapple	100†
Dinner		*Between Meals*	
Wilson's chopped beef	20	3:00 p.m.	
Rice (dry weight)	33	Lemon juice	20
Green beans or	100†	Sugar	20
Mustard greens	50†	8:00 p.m.	
Corn bread or	75‡	Prune juice or	100
Masa bread	75‡	Apple juice	100
Oleomargarine	10		
Fruit cocktail or	100†		
Apple sauce	150		
Apple juice	200		

* Corn grits, 10% moisture; masa mush, 62% moisture.
† Canned drained weight—given on alternate days.
‡ Recipe for corn bread or masa bread:

Masa	100 g (62% moisture) + water, 55 g or		
Corn meal	45 g (10% moisture) + water, 110 g		
Flour	27 g	Baking powder	4 g
Salt	2 g	Shortening	10 g
Sugar	2 g		

Bake about 30 minutes at 450° F.
Cooked weight about 150 g

The unenriched white flour used in making bread was furnished by General Mills through the courtesy of Dr. H. S. Faulkner; the unenriched corn meal and grits by the Quaker Oats Company through the courtesy of Dr. F. M. Peters. The masa was prepared from Guatemalan corn by Dr. N. S. Scrimshaw and associates.

The diets used in these studies are shown in Table I. Masa was cooked as mush or made into bread in three experiments; corn grits and corn bread were used in the other three. The energy value of the diets was adjusted by modifying the sugar and oleomargarine content. In the first two experiments, the subjects were given diets supplying 38 and 31 calories per kilogram of body weight, respec-

* Preparation of masa. Corn was placed in a 5-gallon can and covered with 0.38 to 0.47 per cent lime water. The mixture was heated for 45 to 60 minutes at 94° C, the temperature of boiling water in the locality where it was prepared. The mixture was allowed to stand about 14 hours. The supernatant liquid was decanted and the corn washed 4 to 5 times with water. The treatment thus far results in shedding of the ectosperm, leaving the endosperm and germ softened. Following this, the corn was ground into a fine dough in a commercial mill and then hand ground to the point where it was ready for the baking of tortillas.

tively. In the remaining four experiments, the energy value of the diet was adjusted to furnish approximately 27 calories per kilogram of body weight daily. Vitamin supplements* were administered once daily in the following amounts: 1 mg thiamine, 1.5 mg riboflavin, 5 mg pantothenic acid, 5 mg pyridoxine, 2.5 mg folic acid, and 5 μg vitamin B_{12}. The complete diet for two days was analyzed at intervals for niacin and tryptophan by methods used in previous studies.[6]

The subjects were examined frequently for signs which might be indicative of niacin deficiency. All urine was collected for 24-hour periods in dark bottles containing 5 ml of glacial acetic acid and was kept in the refrigerator. Urine collected over periods of two or four days was pooled and a portion of each pool was adjusted to pH 6.9 and stored in the frozen state. Analyses were carried out with methods previously described[6] for the following constituents: total nitrogen, creatinine, niacin, N^1-methylnicotinamide (N^1-Me), the 6-pyridone of N^1Me (pyridone), quinolinic acid, and tryptophan. Other laboratory tests which were carried out at intervals included gastric analysis, complete blood count, urea nitrogen and sugar in blood, albumin and globulin in serum, glucose tolerance test, basal metabolic rate, electrocardiogram, and liver function tests (cephalin flocculation, serum bilirubin, thymol turbidity and bromsulfalein excretion).

Following the experimental period of low niacin and tryptophan intake, an "adequate" diet was prescribed which furnished approximately 10 mg of niacin and 1000 mg of tryptophan. Estimation of the urinary excretion of niacin and tryptophan metabolites was continued. The response to administration of test doses of 10 and 25 mg of niacin was also determined. Subsequently, the "adequate" diet was supplemented with 10 mg of niacinamide daily and the patients were followed until rehabilitation was essentially complete.

TABLE II

Average Daily Intake of Corn, Niacin, Tryptophan, and Calories of Subjects Receiving Corn Diets

Experiment no. and subject	Weight	Diet	Corn products, dry weight	Niacin*	Tryptophan*	Calories
	kg		*g*	*mg*	*mg*	
1 (E. F.)	47.7	Untreated corn†	87	5.2	183	1785
2 (L. W.)	58.6	Lime-treated corn	76	5.4	196	1800
3 (E. G.)	68.6	Lime-treated corn	76	5.4	196	1945
4 (E. G.)	65.7	Untreated corn	81	4.8	170	1755
5 (O. B.)	69.5	Lime-treated corn	76	4.9	180	1985
6 (O. B.)	71.4	Untreated corn	81	5.1	197	1910

* Determined by analysis of diets and corrected for any food refused.

† The corn used in experiment 1 was obtained in the United States, that used in the other experiments in Guatemala.

RESULTS

The average daily intake of corn, niacin, and tryptophan of the six subjects who received the experimental diets is given in Table II. The calories supplied by these diets are also indicated; 15 to 20 per cent of the calories were furnished by corn products. Although the intake of corn was about 5 grams less in subjects who received the diet containing lime-treated corn than in those who received untreated corn, this difference seems insignificant. Five grams of untreated corn would contribute only about 0.07 mg of niacin to the diet. The amounts of niacin and tryptophan furnished by the diets were determined by analysis and corrected for food not eaten. The intake of these nutrients was remarkably similar for all subjects regardless of the type of corn included in the diet.

* The vitamin and amino acid supplements used in this study were generously furnished as follows: thiamine, riboflavin, pantothenic acid, pyridoxine, niacin, and tryptophan by the Medical Research Department of Winthrop-Stearns Inc. through the courtesy of Mr. Kenneth Smoot; folic acid by the Lederle Laboratories of the American Cyanamid Company through the courtesy of Dr. Stanton M. Hardy; and vitamin B_{12} through the courtesy of Merck & Co., Inc.

The first two experiments were conducted concomitantly; degerminated unenriched corn, which was obtained in the United States, was used in experiment 1, lime-treated Guatemalan corn in experiment 2. While typical pellagra was not induced in either experiment, signs suggestive of niacin deficiency were noted in both subjects at the end of the second month. Subject E. F. developed minimal atrophy of the tongue papillae, heartburn, transient vesicular stomatitis, slight scaling of the skin over the elbows, and pain and numbness of the soles of the feet. Three months after the diet was instituted, she had an acute exacerbation of rheumatoid arthritis. Subject L. W. developed slight atrophy of the tongue papillae, transient dermatosis of the chin, minimal, transient angular stomatitis, heartburn, and decreased menstrual flow with an increased interval between menstrual periods. Signs of niacin deficiency failed to progress in either subject, although the experimental diets were continued for a period of five months.

In both experiments, the combined excretion of N^1-Me and pyridone, the two chief niacin metabolites in urine, decreased to a minimal level after 30 days and remained there for the next 70 days (Fig. 1). The mean excretion during this period was identical in the two studies, 1.4 mg, and consisted of approximately equal quantities of N^1-Me and pyridone. After 100 days, excretion increased slightly in both experiments. This was due, apparently, to an increase in the niacin and tryptophan content of the canned foods used in the diet. Such an increase may account for the failure of either subject to develop typical pellagra.

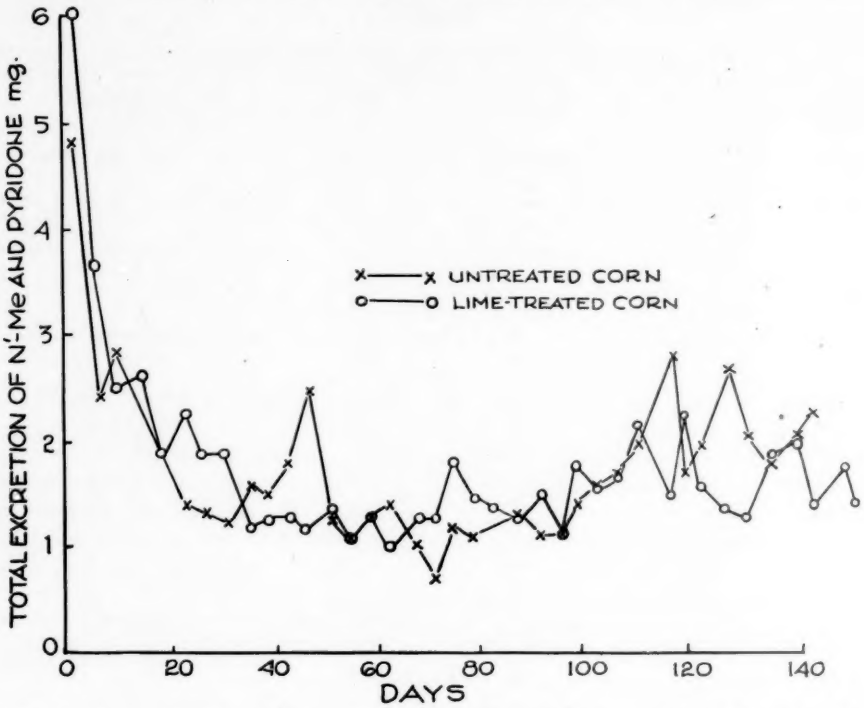

Fig. 1. Urinary excretion of niacin metabolites in experiments 1 and 2 (Subjects E.F. and L.W.).

Subject E. G. served in the next two experiments, receiving the diet containing lime-treated corn first, and after 72 days of rehabilitation, the diet containing untreated corn. Severe, characteristic niacin deficiency developed during both studies after about the same length of time. Symptoms began with soreness of the tongue approximately 30 days

after the diet was instituted and became so severe after 75 days that niacin administration became necessary. Signs of deficiency included mental depression, dermatitis of the elbows, severe glossitis and stomatitis, proctitis, vaginitis, diarrhea, dysphagia, and vomiting. The combined excretion of N^1-Me and pyridone is shown in Figure 2. In both experiments, excretion decreased at approximately the same rate and to about the same level, slightly more than 1 mg daily. At low levels of excretion, approximately equal amounts of pyridone and N^1-Me were found in the urine.

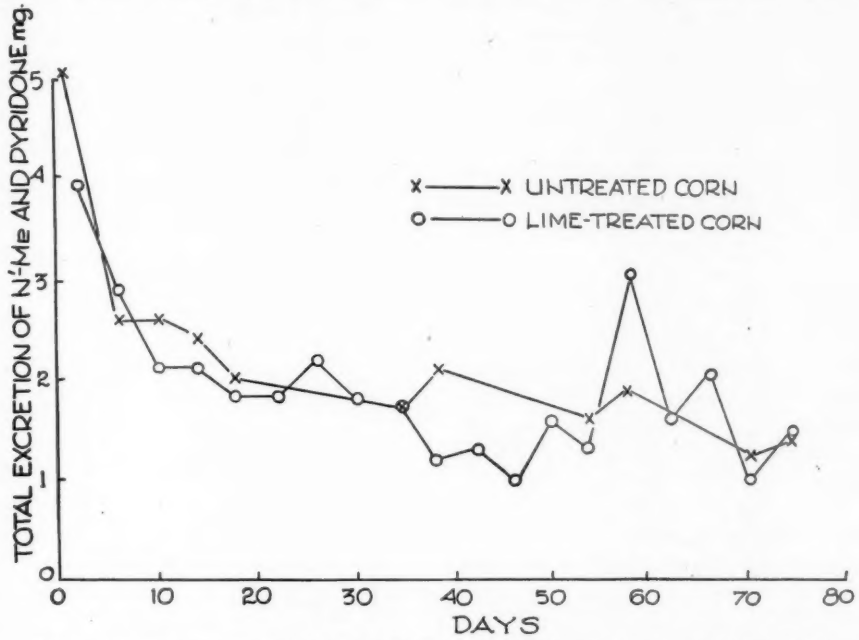

Fig. 2. Urinary excretion of niacin metabolites in experiments 3 and 4 (Subject E.G.).

Subject O. B. was tested with both dietary regimens, receiving the diet containing lime-treated corn in experiment 5, and that containing untreated corn in experiment 6. The rehabilitation period between experiments was of two months' duration. Niacin deficiency developed during both experimental periods, the first lesions appearing after 4 weeks when untreated corn was used, and after 6 weeks when lime-treated corn was administered. The signs of deficiency were similar in the two experiments and included glossitis, stomatitis, heartburn, proctitis, abdominal cramping pain with distention, and dermatitis of the elbows. Niacin was administered after 64 days of each experimental diet. The combined excretion of N^1-Me and pyridone (Fig. 3) decreased in essentially the same manner during both studies, reaching a level of about 1.3 mg after 60 days. Pyridone excretion was slightly greater than N^1-Me excretion throughout both experimental periods.

In all subjects, excretion of niacin, as measured microbiologically, remained unchanged at a level of 0.3 to 0.4 mg daily. Tryptophan excretion averaged 4 to 5 mg in experiments 1, 2, 5 and 6, and varied from 9 to 14 mg in experiments 3 and 4. The excretion of quinolinic acid, a metabolic derivative of tryptophan, remained essentially the same, 3 to 4 mg daily, throughout the period of study in the first four experiments, and decreased slightly, from about 6 mg to less than 4 mg daily, in experiments 5 and 6.

At the end of each of the six experiments, the subjects were tested for the extent of depletion of body niacin stores. In four experiments (1, 2, 5, and 6) the subjects were placed on an

"adequate" diet which contained no corn and furnished approximately 10 mg of niacin and 1000 mg of tryptophan, daily. Excretion of N^1-Me and pyridone was measured for the first 24-hour period of this diet. Subsequent to this, the same diet was supplemented with a test dose of 10 mg of niacinamide and 24-hour excretion of niacin metabolites determined. In experiments 3 and 4, the subject was maintained on the untreated or lime-treated corn diet and received a 10-mg test dose of niacinamide. Excretion of niacin metabolites was determined in the subsequent 24 hours. The corn diet was continued for 3 days without supplementation, following which the diet was changed to the "adequate" diet and tests were carried out as outlined in the other experiments. Results are given in Table III in which findings in five well-nourished control subjects are included for comparison. The excretion of N^1-Me and pyridone on the first day of the "adequate" diet was very low in all experimental subjects, varying from 1.5 to 3.9 mg. Excretion in the controls ranged from 7.0 to 37.5 mg. When 10 mg of niacinamide was added to the "adequate" diet, excretion, again, was very low in the depleted as compared to the control subjects.

In the first four experiments, the subjects received an additional test dose of niacinamide, this time in the amount of 25 mg. Excretion of niacin metabolites varied from 6.2 to 10.9 mg in the subsequent 24 hours. Normal persons excrete approximately 20 mg with this test.

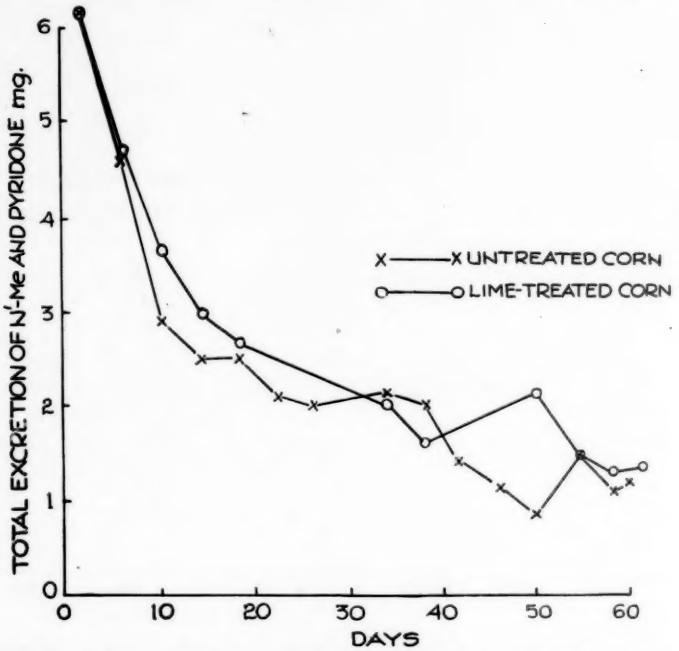

Fig. 3. Urinary excretion of niacin metabolites in experiments 5 and 6 (Subject O.B.).

Rehabilitation of the depleted subjects was followed in all experiments except the first. In experiments 2, 3 and 4, they received the "adequate" diet without supplementation for three days after the test dose, and then the same diet supplemented with 10 mg of niacinamide for 16 days. Excretion during the supplemented regimen did not reach maximum levels until the 9th to the 13th day. In experiments 5 and 6, the subject received the "adequate" diet, which was supplemented with 10 mg of niacinamide, daily, from the second day of rehabilitation. It required 6 days for maxi-

mum excretion to be attained following the experimental period with untreated corn, and 10 days following the period with lime-treated corn. The individuals who served as controls and who were well nourished and received the same "adequate" diet supplemented with

TABLE III

Urinary Excretion of Niacin Metabolites in Response to Increased Niacin and Tryptophan Intake—Subjects Who Previously Received Corn Diets

Experiment no. and subjects	Days on experimental corn diet	Combined excretion of N¹-Me and pyridone	
		"Adequate"* diet (1st 24 hours)	"Adequate"* diet (2nd 24 hours) + 10 mg. niacinamide
		mg	*mg*
1 E. F. (U)†	144	2.8	7.1
2 L. W. (L)‡	152	2.9	3.5
3 E. G. (L)‡	77	2.6§	3.6
4 E. G. (U)†	77	3.9§	3.7
5 O. B. (L)‡	63	1.1	3.5
6 O. B. (U)†	63	1.5	5.6
Controls (5 subjects)	None	7.0–37.5 (mean 19.1)	14.7–29.8 (mean 21.1)

* "Adequate" diet furnished about 10 mg niacin and 1000 mg tryptophan.

† U = untreated corn.

‡ L = lime-treated corn.

§ Received one 10-mg test dose niacinamide 4 days previously while receiving the corn diet. Excretion was 3.1 mg in experiment 3 and 2.1 mg in experiment 4.

niacinamide attained maximum excretion within the first two days.[7] These results suggest that body niacin stores had been seriously depleted in subjects receiving the corn diets. Depletion was essentially the same whether lime-treated or untreated corn was used in the diet.

Certain findings obtained during the period of rehabilitation in experiment 3 are of considerable interest (Fig. 4). Tryptophan excretion began to rise at the end of the experimental period, reaching 18 mg before the "adequate" diet was instituted. Most subjects excrete about 5 mg while receiving the "corn" diet. During the first few days of the "adequate" diet, tryptophan excretion rose to about 40 mg and then decreased to 20–25 mg. The usual excretion of tryptophan during this regimen is 10 to 15 mg. Following institution of the "adequate" diet, and subsequent to the increase in tryptophan excretion, quinolinic acid excretion increased, reaching levels much higher than those usually observed with this intake of tryptophan. Excretion gradually decreased to amounts which are usually noted in subjects receiving this diet. Administration of tryptophan at a later date, and after considerable therapy with niacinamide, was followed by little rise in quinolinic acid excretion. Similar findings were not ob served in any of the other experiments but ave been reported in endemic pellagra.[8]

No changes were observed in any of the other laboratory tests which were carried out at intervals during the corn regimen.

DISCUSSION

Under the conditions of these experiments, no significant differences were observed in the production of niacin deficiency, or in the excretion of niacin or tryptophan metabolites, when lime-treated corn was used in place of untreated corn in diets low in niacin and tryptophan. In these studies, corn products furnished 15–20 per cent of the total caloric intake. In Central America, where corn is lime-treated and eaten as tortillas, and where pellagra is rare, corn products furnish about 80 per cent of the caloric intake. Inclusion of much larger quantities of corn in the diet than were used in these experiments might show differences between the pellagra-producing effect of untreated corn and that of corn treated with lime. If treatment with lime does reduce the pellagragenic effect of corn, one explanation might be the release of "bound" niacin. Scrimshaw[9] found an overall loss of niacin in the process of making tortillas amounting to 20 per cent. In the present study, the niacin content of masa (lime-treated corn) was about 10 per cent less than that of untreated corn. If release of bound niacin is responsible for some pellagra-preventive effect of lime-treated corn, the amount of niacin made available to the human subject would have to be large enough to more than compensate for the overall loss of niacin during treatment. A change in the balance of amino acids in the diet might be another explanation of the low incidence of pellagra in population groups consuming diets high in lime-treated corn, since loss of amino

acids, as well as of niacin, occurs during treatment with lime. Amino acid imbalance has been shown to lead to niacin deficiency in animals.[10]

A simpler explanation for the low incidence of pellagra in Central America might be suggested. The large quantities of corn consumed have been calculated to furnish considerably more than 6 to 7 mg of niacin daily, which amount has been found to approximate the minimal requirement on corn diets low in tryptophan.[6] This explanation may not be tenable, however, since corn furnishes a large percentage of the caloric intake in several areas of the world where pellagra is common, for example, in Rumania, Egypt, and Yugoslavia.[11,12] The niacin intake in these areas would be similar, presumably, to that in Central America, unless the varieties of corn consumed differ significantly in niacin and tryptophan content.

In four of the five experiments in which Guatemalan corn was included in the diet, signs of niacin deficiency appeared earlier and developed more rapidly than in three previous experiments in which unenriched corn obtained in the United States was administered.[6] The diets containing these two types of corn were similar in niacin and tryptophan content. Recent studies have indicated a relationship between niacin and tryptophan intake per unit of body size and the development of pel-

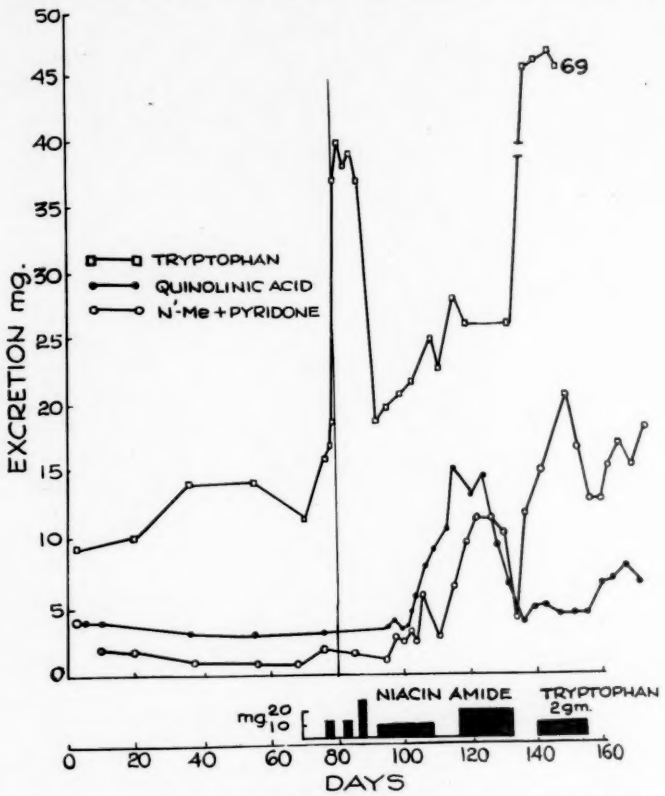

Fig. 4. Urinary excretion of niacin and tryptophan metabolites in subject E.G. (experiment 3). The diet during the 80-day experimental period contained lime-treated corn and furnished 5.4 mg niacin and 196 mg tryptophan. The diet from the 81st to 154th day furnished approximately 10 mg niacin and 1000 mg tryptophan. Supplements of niacinamide and tryptophan were administered as indicated.

lagra.[1] The intakes of niacin and tryptophan per kilogram of body weight of subjects who received Guatemalan corn and of those who received corn obtained in the United States are shown in Table IV. Two of the three subjects who developed pellagra while receiving United States corn had a slightly higher intake of tryptophan, and one a slightly higher intake of niacin per kilogram of body weight than did the subjects who developed severe niacin deficiency while receiving Guatemalan corn. These small differences may explain the earlier appearance of niacin deficiency when Guatemalan corn was given. However, Rose[13] found that the tryptophan requirement was not related to body size. The total daily intake of tryptophan was essentially the same in subjects receiving the two types of corn. It is possible that caloric intake may have influenced findings. With the Guatemalan corn diet, the four subjects who developed niacin deficiency at an early date received fewer calories per kilogram of body weight than did subjects who developed severe niacin deficiency during regimens containing United States corn. In the latter group, the subject (L. M. G.) who received the lowest caloric intake developed signs of deficiency more rapidly than did the other two subjects. It is conceivable that the availability of more energy from nonprotein sources in the experiments with corn obtained in the United States may have spared some tryptophan for conversion to niacin compounds.

Another explanation of the difference in findings with the two types of corn seems worthy of consideration. The corn obtained from Guatemala was whole corn, while that obtained in the United States had been subjected to the usual milling process and hence contained little of the germ or bran layer. Whole corn may contain one or more substances, not present in milled corn, which might influence the development of pellagra. Whether such substances may represent the long-postulated "toxic" or inhibitory factor in corn, or whether a difference in amino acid content of whole as compared to degerminated corn may lead to amino acid imbalance, remains to be investigated.

The excretion of unusually large amounts of tryptophan prior to therapy, and of both tryptophan and quinolinic acid following tryptophan administration, in one subject with experimental pellagra merits comment. A previous study of endemic pellagra, by Sarett and Goldsmith,[8] reported similar findings. In two patients with pellagra, tryptophan excre-

TABLE IV

Average Daily Intake of Niacin, Tryptophan, and Calories per Kilogram of Body Weight of Subjects Receiving Diets Containing Guatemalan and United States Corn (in Relation to the Development of Niacin Deficiency)

Experiment no. and subject	Intake per kilogram of body weight			Signs of niacin deficiency	
	Niacin	Tryptophan	Calories	Time of appearance after beginning experiment	Severity of deficiency
	mg	*mg*		*days*	
			Guatemalan Corn		
2 (L. W.)*	0.09	3.3	31	50	Mild
3 (E. G.)*	0.08	2.8	27	26	Severe
4 (E. G.)†	0.07	2.6	27	31	Severe
5 (O. B.)*	0.07	2.6	29	43	Severe
6 (O. B.)†	0.07	2.8	27	27	Severe
			United States Corn		
1 (E. F.)	0.11	3.8	38	58	Mild
(L. M. G.)‡	0.07	2.7	30	52	Severe
(H. K.)‡	0.08	3.3	37	56	Severe
(R. C.)‡	0.10	4.3	38	52	Severe

* Lime-treated corn.

† Untreated corn.

‡ Subjects reported in detail in previous study (Reference 6).

tion was high prior to therapy. In one, administration of L-tryptophan was followed by a large increase in tryptophan excretion, and a marked rise in quinolinic acid excretion subsequent to this. In the other, unusually large amounts of quinolinic acid were excreted following administration of tryptophan. One explanation of these findings might be that niacin-containing enzymes are required for the conversion of tryptophan to quinolinic acid, and to niacin compounds, and that these enzymes were exhausted in severe depletion.

SUMMARY

Four subjects were maintained for six experimental periods on corn diets low in niacin and tryptophan. In three experiments, the corn was treated with lime prior to incorporation in the diet; in three, the corn was untreated. In two experiments with each type of corn, the subjects developed typical niacin deficiency beginning 4 to 6 weeks after the diet was instituted. In the other two studies, mild signs suggesting niacin deficiency appeared after about two months but failed to progress. Urinary excretion of N^1-methylnicotinamide and the 6-pyridone of N^1 Me decreased to approximately the same minimal levels after the same length of time during both dietary regimens. Likewise, excretion of tryptophan and quinolinic acid differed little during administration of the two types of corn. Under the conditions of these experiments, lime treatment of corn failed to influence the production of experimental pellagra.

Excretion of quinolinic acid and tryptophan was unusually high during the early rehabilitation period in one subject. Similar findings have been encountered previously in endemic pellagra.

Pellagra was induced more rapidly with diets containing Guatemalan whole corn than with diets containing unenriched milled corn obtained in the United States. This observation might be explained by the presence of substances in the bran or germ layer of corn which influence the pellagragenic effect of this cereal. The simplest explanation for the high incidence of pellagra in association with corn diets is their low niacin and tryptophan content. Findings reported here, and others published previously, suggest that additional factors may be operative.

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Lysine and Tryptophan Content of Proteins and Their Utilization for Human Growth

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MITCHELL,[1] in 1950, presented evidence that amino acid analyses of mammalian carcasses might prove to be an effective method for estimating the requirements of some, if not all, of the essential amino acids for growth. In that same publication one of us[2] presented data on the amino acid requirements of infants obtained under uniform and controlled dietary conditions. From these data calculations of a growth pattern of amino acid requirements were obtained by assigning value as unity to the tryptophan need (Table I). A similar calculation of the amino acid content of mammalian tissues recorded by Mitchell showed that there exists good agreement of growth needs and tissue amino acid patterns. This agreement is particularly good for the lysine/tryptophan (L/T) and methionine/tryptophan (M/T) ratios of muscle proteins which constitute approximately 75 per cent of the infant body proteins.

In the light of these data and the lysine and tryptophan content of foods commonly employed in the feeding of infants,[3] it was felt worth while (a) to re-examine the nutritional value in infants of various dietary proteins in terms of their lysine/tryptophan (L/T) ratios, and (b) to explore the nutritional effects of graded lysine supplements to dietaries having L/T ratios below 6.3 (that of muscle proteins). The present report records our findings on these two points.

TABLE I

Pattern of Amino Acid Needs of Infants Compared to the Composition of Mammalian Tissues*

Amino acids	Male infant needs (Albanese[2])		Mammalian tissue patterns (Mitchell[1])		
	mg/kg/day	Growth pattern	Muscle proteins	Viscera	Plasma proteins
Tryptophan	30	1.0	1.0	1.0	1.0
Lysine	180–210	6.0–7.0	6.3	5.3	6.2
Methionine	85	2.8	2.5	2.0	1.1
Isoleucine	90	3.0	4.5	3.3	2.0

* Growth and tissue pattern figures were obtained by assigning a value of unity to tryptophan.

EXPERIMENTAL

Utilization of Various Protein Products by Infants

In previous publications we reported measurements of the biological value of some protein products in infants in terms of nitrogen retention, body weight change, and blood protein levels.[4–7] In these studies, all products were fed in synthetic formulae providing 3.5 g of protein (N × 6.25) and 100 calories per kilogram per day. These formulae had a caloric density of 1 calorie per ml, and a percentile caloric distribution of protein, 15; carbohydrate, 50; and fat, 35. All the known vitamin and mineral requirements were supplied at or above recommended levels. Vitamin-fortified evaporated milk formulae were fed in the pre- and post-control periods at isonitrogenous and isocaloric levels with the test diets.

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The relative content of the critical amino acids of the evaporated milk and test formulae, as compared to the measured amino acid needs of infants (2–9 months), is shown in Table II. It will be noted that the recorded L/T ratio for evaporated milk is slightly lower than that reported for whole cow's milk.[8] This difference may be due in part to the destruction of lysine during processing of the milk. Since the proteins of all products tested contained approximately 1 per cent tryptophan and were fed at the uniform level of 3.5 g of protein/kg/day, the diets all provided approximately 35 mg of tryptophan/kg/day. This is an amount of tryptophan which we have found to cover the measured infant need of 30 mg/kg/day.[9]

TABLE II

Amino Acid Pattern of Infant Needs Compared to Some Protein Products*

Amino acids	Infant needs	Evaporated milk	Casein (Amigen)	Lactalbumin (Edamin)	Beef muscle digest	Bovine plasma (Travamin)	Wheat gluten
Tryptophan	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Lysine	6.0–7.0	4.3	3.9	3.5	5.9	6.9	2.0
Methionine	2.8	2.1	2.0	1.1	2.6	1.7	3.0
Isoleucine	3.0	3.6	3.8	2.6	3.6	2.7	3.3

* These relative values were calculated from the best amino acid analytical data available for the products employed. In most instances these data were found to check with triplicate analyses in our laboratory by methods published by the author and his associates.

In order to arrive at a relative utilization value of the test protein products the following formula was applied to our results:

$$P = BW \times N/1000$$

where P = protein utilization, BW = body weight change in g/day, and N = nitrogen retention in mg/kg/day.

The coefficient of utilization of the test products, Pr, is then expressed as the numerical value of the ratio: P test protein formula/P evaporated milk formula; or $P_r = P_{tp}/P_{em}$. Expression of the bioassay results in this manner has several advantages. First, it equalizes disparities between body weight changes and nitrogen retention values which often arise in infants from transpositions of body fluid compartments. Secondly, it directly relates increments in nitrogenous tissue to qualitative amino acid differences of the test nitrogenous moiety of the diets. And lastly, it provides a simple numerical comparison of the test substance with a standard infant food—evaporated milk.

An example of such calculations for a male infant, W. M., weighing 3,327 g at 1.5 months of age follows: consumption of a standard evaporated milk formula at the average level of 3.11 g N/day for two weeks resulted in an average body weight change of 23 g/day and a nitrogen retention of 138 mg/kg/day. Thus, $P_{em} = 23 \times 138/1000$, or 3.17. During the subsequent two weeks this infant consumed an isocaloric bovine plasma digest formula at the average rate of 3.12 g N/day. This dietary resulted in an average body weight change of 40 g/day and a nitrogen retention of 180 mg/kg/day. Thus, $P_{tp} = 40 \times 180/1000$, or 7.20. Hence, the coefficient of utilization (P_r) of the bovine plasma digest formula in this single assay is 7.20/3.17, or 2.3. The average P_r of ten such bioassays in infants 1.5 to 9.0 months of age was 1.82.

The details and results of this comparison are collected in Table III. These data clearly show that bovine plasma and beef muscle digest are more effectively utilized for growth by the infant than cow's milk proteins or their products. Reference to the data contained in Tables I and II leads to the impression that greater biological availability of the bovine products may be associated with the approaching coincidence of their lysine and tryptophan content with (a) the lysine/tryptophan ratio needs of the infant and (b) the lysine/tryptophan ratio of the predominant infant tissues. It is also clear from our studies that the possible correlation of the L/T ratios of protein substances and their utilization in infants prevails only for products which have overall amino

acid patterns not too greatly different from tissue proteins and which provide 30 or more mg of tryptophan per 3.5 grams of protein (N × 6.25). A substance like gelatin which has an L/T of infinity by virtue of its lack of tryptophan could not be expected to have exceptional nutritional values unless its primary amino acid deficiencies and imbalances are corrected. As a matter of fact, unpublished studies in our laboratory have shown that the amino acid imbalance of gelatin is such that its nutritive value cannot be greatly improved by supplementation of its deficiencies by crystalline amino acids.

The following observations also made during the course of these studies seem particularly worthy of note in the development of our thesis:

Supplementation of the casein digest (Amigen ®) with 1 per cent of DL-methionine was found not to improve the utilization of this product.[10] In view of the carcass method concepts, this result could have been anticipated since the methionine/tryptophan (M/T) ratio of Amigen closely approximates the average for mammalian tissues (Table II). Also, in retrospect, our failure to improve the utilization of the bovine plasma digest[7] by the combined addition of 1 per cent DL-isoleucine and 0.6 per cent DL-methionine can also be ascribed to the fact that the isoleucine/tryptophan and methionine/tryptophan ratios of the commercial product (Travamin ®) are in approximate agreement with those prevailing in mammalian tissues (Tables I and II).

Effects of Lysine Supplementation on Protein Utilization

A careful consideration of the foregoing and, in particular, the data contained in Table III eventually led to the thought that infant utilization of milk proteins could be improved by additions of lysine. Calculations indicated that L-lysine supplements of 50–100 mg/kg/day might prove effective with infants receiving 3.5–4.0 g of total milk proteins/kg/day. Lysine supplements of this order of magnitude would increase the L/T ratio of the evaporated milk formulae from 4.3 to 6.0–8.0.

TABLE III

The Lysine/Tryptophan (L/T) Ratio of Various Protein Products and their Utilization in Infants*

Protein source	L/T of product	Subjects		Average coefficient of protein utilization (P_r)
		No.	Age (mos.)	
Lactalbumin digest (Edamin)	3.5	6	5.0–11	0.89
Casein digest (Amigen)	3.9	6	5.0–11	0.95
Evap. milk	4.3	38	1.0–27	1.00
Beef muscle digest	5.9	5	2.5–8.0	1.43
Bovine plasma digest (Travamin)	6.9	10	1.5–9.0	1.82
Wheat gluten	2.1	6	3.0–8.0	0.52
Wheat gluten plus 2.0–3.0% L-lysine	5.0–6.0	6	3.0–8.0	1.05
Evap. milk plus 2.0–4.0% L-lysine	6.0–8.0	8	1.0–27	1.75
Amigen plus 2.0–4.0% L-lysine	6.0–7.0	3	6.0–9.0	1.65

* All diets were fed at the rate of 3.5 g of protein (N × 6.25) and 100 calories per kg per day.

Preliminary studies on 15 infants previously reported in this Journal[3] showed that lysine supplementation of the diet of infants with poor appetites with 100 mg/kg/day of L-lysine was associated in most instances with a marked increase in daily nitrogen retention and body weight, as well as significant increases in blood protein levels.

Subsequently, it appeared worth while for basic and practical reasons to define more accurately the minimum limits of effective lysine supplementation. In this phase of our investigations, 13 infants, 1 week to 14 months of age, were studied as previously described, with the exception that the lysine additions were made as follows: After a pre-control period of 3 or more weeks, L-lysine was added to the formulae at the rate of 250 mg of lysine/day for infants under 4 kg (8.8 lb) and 500 mg of lysine per day for infants over 4 kg. This initial amount of daily lysine supplement was

continued for the succeeding 3 to 5 weeks. In this manner the changes in body weight occurring during the lysine period had the effect of altering the rate of supplementation. The effect of the lysine supplement was determined from changes in the slope of the body weight curves. Whenever possible this procedure was repeated and in some instances the lysine supplement level was adjusted as previously noted after post-control periods of 3 to 4 weeks' duration.

The lysine supplement was contained in a mixture of vitamins and minerals which brought the intake of these dietary elements to or above recommended levels. In order to maintain a uniform vitamin and mineral intake during these investigations, a lysine-free form of the product was added to the infant formulae throughout the pre- and post-control periods.*

Typical studies illustrating the application of these procedures to three infants whose combined ages cover the first year of life are shown in Figure 1. It will be noted that good body weight responses were obtained in infant B. P. with 250 mg of L-lysine per day up to a weight of 9 to 10 lb (Chart I). In the third supplemental period a delayed and small weight effect was obtained with 250 mg of lysine per day.

In contrast, infant B. R. (11.5 lb) showed a significant weight change when 500 mg of L-lysine were added to his diet (Chart II). This response carried over into the first week of the post-control period, after which time the weight curve flattened out. Examination of Chart III shows that 500 mg of L-lysine per day also induced an accelerated body weight gain in a 19-lb infant (P. F.). That the biological changes induced by lysine are symmetrical is indicated by the fact that supplementation was generally associated with positive changes in development curves of the Wetzel grid type.

Because the lysine supplements employed constitute less than 1 per cent of the total nitrogen intake of these infants the observed changes cannot be reasonably ascribed to the increased nitrogen intake arising from the lysine.

Our observations on nine other infants showed an average 34 per cent greater rate of weight gain during the lysine supplement period than in the pre-control period. This result was achieved despite the fact that this group included one underweight infant of poor appetite and two infants of normal weight and appetite who showed no increase in rate of weight gain during their lysine supplement periods. An average 9 per cent greater weight gain was also observed in the post-control periods. This would appear to be due to a metabolic carry-over effect. The blood protein changes in general paralleled the growth change.

Examination of these and previously published data (a total of 27 infants) indicates that improved nutrition in terms of body weight change (a commonly accepted criterion of nutritional state) occurred when L/T ratios of 6.0–7.0 were maintained by lysine additions to diets which provided a minimum of 100 calories and 3.5 g of protein per kilogram per day. Increased milk protein utilization as previously defined under these conditions is shown in Table III. It will be noted that lysine-reinforced milk proteins attain a degree of utilization comparable to that of bovine muscle and plasma digests. Our data further indicate that the nutritive value of a casein digest (Amigen), a product often employed in the feeding of milk-allergic infants, may also be improved by additions of lysine (Table III).

We have not had the opportunity as yet to determine the effect of lysine supplementation on the utilization of soy bean preparations often employed for the feeding of allergic infants. However, a consideration of the reported low L/T values (1.7–2.0) of these products suggests that some nutritional value might be gained by lysine supplementation of the diet

* These products were kindly made available to us by Dr. Edward R. Neary of the White Laboratories, Inc. The lysine-containing preparation (Lactofort®) had the following composition: L-lysine (as Darvyl DuPont), 500 mg; vitamin A, 3750 USP units; vitamin D, 1000 USP units; thiamine, 0.75 mg; riboflavin, 1.25 mg; pyridoxine, 0.75 mg; niacinamide, 7.5 mg; Ca pantothenate, 7.5 mg; vitamin B_{12}, 2.5 μg; folic acid, 0.25 mg; ascorbic acid, 75 mg; iron, 7.5 mg; calcium, 130 mg.

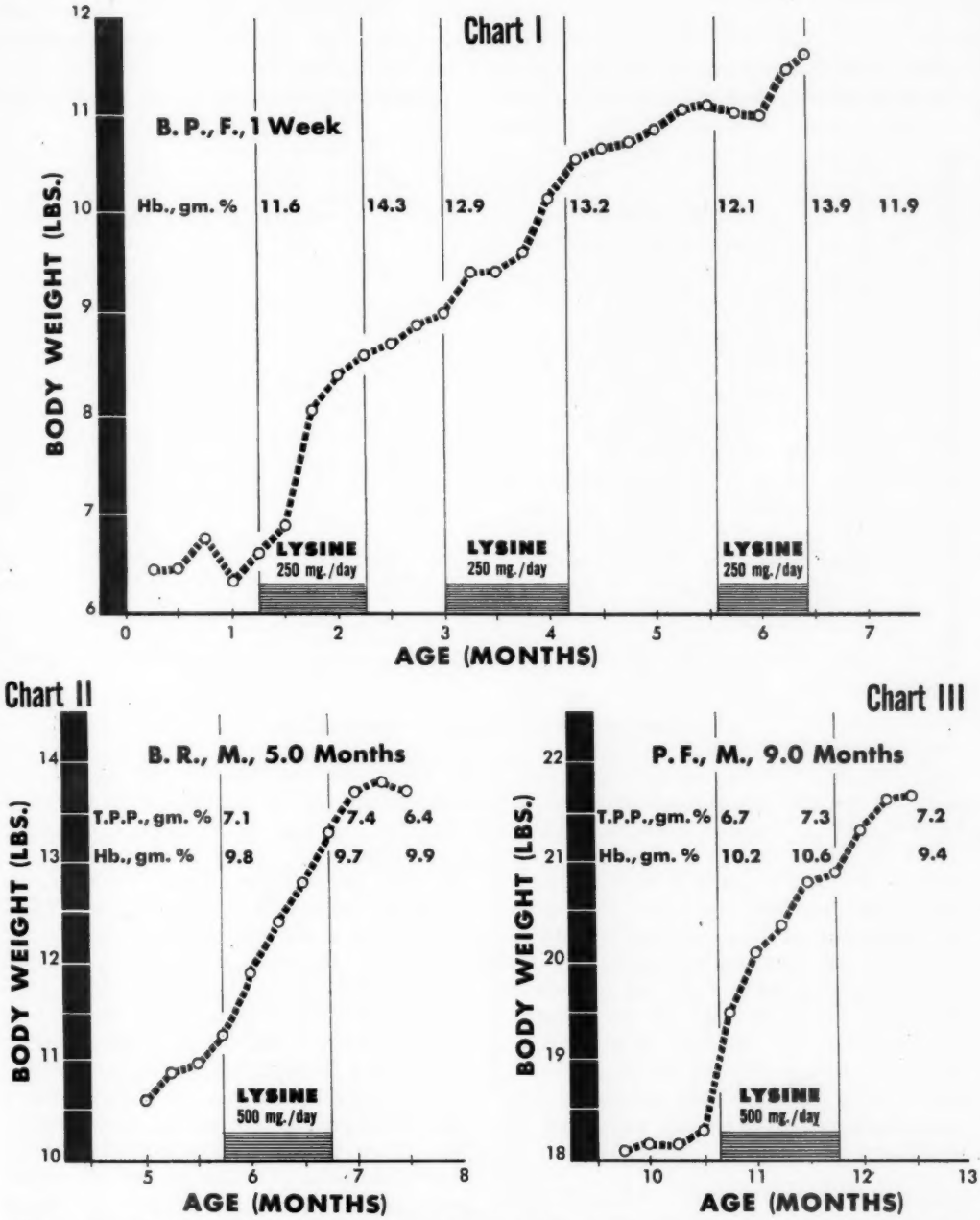

Fig. 1. The effect of lysine supplements on body weight and blood proteins of three nutritionally substandard infants covering the first year of life.

of infants maintained on the commercially available soy bean products.

DISCUSSION

The observations presented in this report clearly suggest that the carcass method for the estimation of the amino acid needs has some applications in human nutrition. One of the most promising of these applications may be based on the apparent relationship of the L/T ratios of proteins and their utilization. Accordingly, it was of some interest to find on algebraic analyses that the utilization coefficient of dietary proteins (Pr) as previously defined is linearly related to the lysine/tryptophan (L/T) ratio of six protein products by the equation,

$$P_r = 0.28\ (L/T) - 0.15$$

A graphic representation of the fit of the experimental data to the line of this equation is shown in Figure 2.

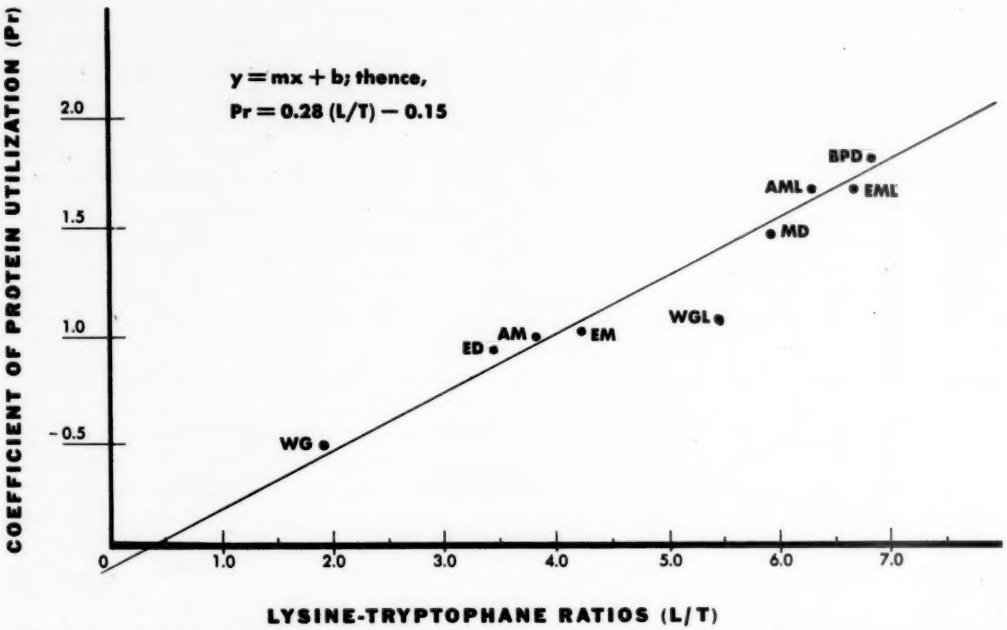

Fig. 2. Algebraic relationship of the L/T ratios of some protein products and their coefficient of utilization in infants. The products tested are designated as follows: WG, wheat gluten; ED, Edamin; AM, Amigen; EM, evaporated milk; WGL, wheat gluten with lysine; MD, meat digest; AML, Amigen with lysine; EML, evaporated milk with lysine; BPD, bovine plasma digest (Travamin).

Further support for this relationship is derived from the findings that the increase in the value of P_r for L-lysine-reinforced evaporated milk or casein digest formulae is in good accord with that expected from the augmented L/T values of 6.5–7.0.

Modification of the L/T of a wheat gluten diet from 2.0 to 5.5 by additions of lysine was found to improve its nutritional value (Table III) to that of the milk protein diets (L/T 3.5 to 4.3).[6,11] However, this improvement fell short of that expected from the equation (Fig. 2). At first sight this anomaly seemed to point to the existence of a limiting level of some other essential amino acid or acids in wheat gluten. Reference to Table II shows that methionine and isoleucine are probably not implicated in this nutritional shortcoming of lysine-reinforced wheat gluten. However, consideration of the recent investigations of Williams and co-workers,[12] which lend considerable support to the carcass theory of amino acid requirements for growth in rats, also suggests that the observed nutritional limitation of

wheat gluten probably arises from an amino acid imbalance created by the presence of excessive amounts of glutamic acid.[13] The importance of these possible relationships in attempts to improve the nutrition of populations of underdeveloped world areas whose dietaries are comprised largely of wheat and other cereal products is obvious.

TABLE IV

Comparison of Amino Acid Needs of Infants and Amounts Provided by Normal Breast Feeding

Amino acid	Estimated daily minimal needs 5–12 months	Daily amino acid intake calculated for normal breast feedings* 3 mo	6 mo	9 mo
Tryptophan	30	49	37	34
Lysine	180–210	150	113	103
Methionine	86	46	35	32
Isoleucine	90	120	90	83
Phenylalanine[14]	170–205	123	92	85

* The amino acid intake was estimated from available data on the daily human milk intake of breast-fed infants and the amino acid analyses data of breast milk.[5,9]

A relevant problem in these low economic areas concerns the nutritional limitations of restricted human breast feeding of infants. This form of breast feeding is not to be confused with that practiced in this country or other high economic areas. In our country generous supplements of vitamins, cow's milk formula, and prepared meats are introduced very early in the diet of breast-fed infants. This current, empirically-derived practice amply supports recently obtained objective evidence[9] which suggests that even on the most liberal intake estimates, breast milk alone provides a very meager margin of safety during the early months of life and that it does not provide an adequate quantity of some essential amino acids in the latter part of the first year (Table IV) if fed as the chief source of nutrients. It is clear from these data that restricted breast feeding as it prevails in many world areas is not likely to provide for optimal nutrition of infants. The data in Table IV suggest that this undernutrition may be due in part to the relative lack of lysine (and probably other amino acids) (Table I).

Obviously, to have practical significance such far-reaching deductions require proof of greater statistical weight than can be derived from our short-term studies on the amino acid needs of infants. Fortunately, such evidence is in part afforded by the investigations of Su and Liang[15] on the growth and development of Chinese infants in different nutritional environments. A graphic analysis of their data on 949 infants on Wetzel Baby Grids[16] would seem to fully warrant the following comments of these investigators: "The slow growth in the latter part of the first year may probably be explained on a nutritional basis. Parents usually do not take our advice that breast feeding meets the nutritional requirements of a baby for six months, at most, while in the latter half of the first year, supplementary feedings should form the most important part."[15]

In this connection, the original report on kwashiorkor in children of the Gold Coast Colony by Williams[17] ascribes the occurrence of this malignant malnutrition, in part, to native tribal customs of infant feeding. That is, babies are breast-fed until the next child is born—a period rarely less than two years. Supplementary feedings consist of nothing more than preparations of corn known as *arkasa* and *kenki*. Recent observations by other investigators suggest that qualitative and quantitative amino acid deficiencies arising from this practice of restricted and poorly supplemented breast feeding may be an important etiologic factor in this malignant malnutrition syndrome. The implication of amino acids is further emphasized by the recent report of Close[18] which revealed that the breast milk of African women contained appreciably smaller quantities of methionine, threonine, and lysine than the breast milk of European women.

SUMMARY

Data have been presented which support the conclusion that the carcass analysis procedure proposed by Mitchell may be a valid method for evaluating the human growth needs for some "essential" amino acids. In particular, the available evidence indicates that the utilization of dietary proteins increases as their lysine and tryptophan content approaches

that of muscle tissues. This concept gains further validity from the fact that the nutritional value of some protein products with low lysine/tryptophan values can be enhanced by small additions of lysine. In milk protein products this increased utilization approached that of bovine muscle and plasma digests and appears to be a linear function of the augmented lysine/tryptophan values. Lysine supplementation of wheat gluten increased its nutritive value to that of milk proteins. The practical implications of these findings in the protein nutrition of infants of population groups of low economic status are discussed. Reference is made to the possible role of amino acid deficiencies in malignant malnutrition (kwashiorkor) of infants maintained on restricted or poorly supplemented breast feeding.

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Relation between Caloric Intake, Body Weight, and Physical Work:

STUDIES IN AN INDUSTRIAL MALE POPULATION IN WEST BENGAL

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IT HAS been stated—or implied—by many workers that the regulation of food intake functions with such flexibility that an increase in energy output due to exercise is automatically followed by an equivalent increase in caloric intake. This view, usually accompanied by a minimization of the energy expenditure due to exercise, has often led to the disparagement of physical activity as a factor in weight control. The fallacies inherent in such an attitude have been discussed previously.[1,2] It has been shown that when rats are exercised for increasing durations on a treadmill, intakes vary linearly with exercise only within certain limits of activity.[3] Below this level, in what can be termed the "sedentary" range, a further decrease in activity is not followed by a decrease in food intake but, on the contrary, by a slight but significant increase. The animals increase both their weight and fat content. Enforced total inactivity in the rat can even lead to a considerable degree of obesity.[4] It has also been shown that spontaneous inactivity is a major factor in the development of genetic obesity in the mouse.[5] Enforced exercise decreases weight gain even when food is available *ad libitum* in other types of animal obesity.[6,7] These findings appear related to the well-known practice among farmers to restrict the activity of animals (e.g., hogs, geese) which they want to fatten.

It appeared of interest to see whether these experimental results could be extended to man. The availability for study of a large population of male subjects demonstrating extreme differences of physical activity made it possible to attempt to correlate work with food intakes and body weight.

SUBJECTS

The subjects constituted a sample of 213 workers out of a total of 800 on whom height and weight data were obtained. All were employees (or, in the case of the "stall holders" or bazaar shopkeepers, licensees) of the Ludlow Jute Co., Ltd., at Chengail, West Bengal. The mill is situated on the Hooghly (Ganges) about 20 miles south of Calcutta, and it and the "labor lines" (workers' quarters) have been described by Chernin[8] on the occasion of his studies of parasitic infestations in the population of this mill. The total number of workers is in the neighborhood of 7,000.

The study was carried out on individuals 5 feet 2 inches to 5 feet 4 inches tall, the most usual range among the male workers of the mill. They were reasonably healthy individuals, free of obvious signs of malnutrition, of which those most frequently encountered in this population were anemia, angular stomatitis and other riboflavin deficiency symptoms, and ocular and skin anomalies probably referable in part to vitamin A deficiency.

The subjects, some 50 per cent of whom were

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indigenous Bengalis, also included persons from other parts of India as well, in particular Orissa, Madras, Bilaspuri (Central Province), Bihar and United Province. Bengalis, although almost equally divided between Hindus and Moslems in the workers' population as a whole, were preponderantly Hindu in this sample, as were workers from other provinces. These men were engaged in 16 occupations, which involved varying degrees of physical activity. In addition, the large clerical group was split into 4 subgroups depending essentially on the distance from their homes to the mill. Unemployment is widespread among people of this degree of education in Bengal and, as a result, "babus" (clerks) will commute considerable distances to a place of work—in this case, because of the lack of roads fit for buses, on foot and more exceptionally on bicycles.

METHODS

Food intakes were obtained by exhaustive dietary interviews using a pre-established questionnaire based on cross checks (by meals and by foodstuffs). The interviews were generally conducted in Bengali or Hindustani. In a few cases, interviews were conducted in English or by way of a reliable interpreter with individuals who spoke Telegu. Because most individuals interviewed lived away from their families, had little facility for storing food, and had a very uniform diet, additional checks could be obtained by questions on the amounts of food bought daily and weekly and on the amount of money spent on edibles (lists and prices of foods locally purchasable in one bazaar and in the village were obtained and checked). Repeated interviews and cross checks on individuals chosen at random, conducted at several weeks' intervals gave good agreement. A few random checks by direct weighing also confirmed the general reliability of the data; in fact, the extraordinary uniformity of the diet from day to day, and the monotony of living conditions gave a much higher degree of reproducibility than could be obtained in a Western population.

The results were analyzed using the Indian Food Composition Table established by the Coonoor Nutrition Laboratory,[9] and the following values calculated for each individual and averaged for each group (with standard deviations): animal protein, vegetable protein, total protein, fat, carbohydrate, calories, thiamine, riboflavin, niacin, and vitamin C. Activity was evaluated by a combination of methods. A detailed observation of each occupation was undertaken, with particular care given to "micro-schedule" (pauses, intervals between movements, etc.). Secondly, a thorough analysis conducted by an efficiency engineers' firm was available.

In this survey, "physical demand" was characterized on the following basis: "Physical demand is defined as the amount and continuity of physical endurance needed and for which allowances must be made to compensate for the degree of fatigue involved. Work necessitating awkward bending, stretching, reaching and other unusual postures must be recognized and rewarded. Also, the muscular effort required in excessive walking, standing on hard floors...must receive rating attention." A number of examples were used to illustrate the attribution of ratings: e.g., hand lifting weight up to 10 lb—infrequently, none, frequently, very low; up to 50 lb—infrequently, low, frequently, medium; up to 75 lb—infrequently, high, frequently, very high. Eighty-one occupations were analyzed in this fashion.

Oxygen consumption and carbon dioxide production data pertaining to a number of occupations in the jute industry as obtained by Sen, Gupta, and Ferris[10] were also available. While it was obviously impossible to calculate energy expended as work as such, the foregoing information seemed to permit a classification of the occupations in order of increasing caloric cost. At any rate, broad classes of activity could be differentiated with a reasonable certainty as to differences in energy cost. The following classes and groups were studied (ranged in order of increasing activity):

1st Class: *sedentary.* Thirteen *stall holders,* constituting a group of extraordinarily inert mode of life, who sat at their shop all day, except for one day a week and on occasional buying forays; 8 *supervisors* who lived on company premises and who either sat at desks or confined their walking to the precincts of one department; 22 *clerks* (called clerks I in Table I) who lived on company premises and who did not engage in any sport.

Their walking was confined, during most of the week, to company premises.

2nd Class: *light work.* Three groups of *clerks*, 13 in group II and 10 in group III, who were differentiated by the fact that group II and III lived outside of the mill compound, with group II commuting at least 3 miles and group III at least 6 miles. Group IV engaged in athletic activities (soccer) besides commuting. There was also a group of 22 *mechanics*, whose job was relatively standard except inasmuch as they did not have many power aids at their disposal.

3rd Class: *medium work. Drivers* (10 men) drove either electric tractors or steam locomotives, and did some coupling and uncoupling of wagons. The other groups, 10 *winders*, 10 *weavers*, and 10 *bagging twisters*, were composed of individuals driven at a steady pace by the nature of the machines they were operating. A number of operations involving some strength (lifting of rolls, pushing on large levers, etc.) were involved.

4th Class: *heavy work.* Eleven *mill waste carriers* who carried small bales of 50 to 75 lb. The work of *pilers* (10 men) and *selectors* (10 men) involved the handling of heavy bales (150 to 200 lb) at intervals and on relatively short stretches, as well as the opening of bales, lifting of fractions thereof, etc.

5th Class: *very heavy work. Ashmen* and *coalmen* (15 men) shoveled ashes and coal in tending furnaces. The work of *blacksmiths* corresponded to the classic occupation without power aids (15 men). *Cutters* (10 men) swung heavy cleavers and hacked steadily at jute fibers, a particularly hard and tiring procedure. Finally, 19 carriers were divided between 9 *Bojha carriers* (190 lb per man) and 10 *bale carriers* (400 lb for 3 men).

Additional information which was obtained included: height and weight (by means of portable scales and height-measure with sliding headpiece), religion and caste, income, numbers of dependents, regular payments on loans, amount of money sent home weekly, amount of money spent on items other than food, and educational level. Age had to be estimated, from questions and from physical appearance, as the absence of a system of registration of births results in a general ignorance of one's own age, even among clerks and supervisors.

RESULTS

The relation of caloric intake to physical activity is given in Figure 1. Differences in caloric intakes between classes were statistically significant; differences between groups within classes generally were not. Caloric intakes were adequate to satisfy hunger, and for the "light work" and more active classes agreed well with the caloric requirements which could be calculated on the basis of the FAO Committee report.[11] The major proportion of calories was derived from carbohydrates in all groups, with an increasing proportion of fat in the higher intake groups (stall holders and supervisors, pilers, selectors, and "very heavy work" class), all of which consumed more than 50 g of fat per day (Table I). Protein intakes were at or above 70 g per day, but the major part of the protein (at least three-fourths) was derived from vegetable sources. Only two men were total vegetarians, but the amount of animal products generally consumed was so low that for several groups (mill waste carriers, winders, weavers, mechanics) the daily intake of animal protein was on the average of less than 10 g. It may be noted that the weavers and mechanics were among the better paid groups, so that their lower animal protein intake did not represent a more stringent economic necessity. Weavers were predominantly Moslems, so that religious practices were not involved in this avoidance of animal products. Clerks had on the whole the largest intake in animal proteins, in spite of their being predominantly Hindu with an appreciable proportion of Brahmins among them.

Daily thiamine intakes were adequate (above 1.4 mg on the average for all groups, and generally in the 1.6–1.9 mg range, as were niacin (above 12 mg) and vitamin C (30–60 mg range), but riboflavin intakes were very low (generally in the 0.6–1 mg range) (Table I). Carotene-vitamin A and calcium levels were low but were difficult to estimate on a group basis because of the widespread practice of chewing lime-treated green leaves wrapped around betel nuts.

Caloric intakes were not correlated with income (clerks, mechanics and weavers had higher incomes than men in the "heavy work" and "very heavy work" categories); nor were caloric intakes correlated with religion or caste. The differences in religion had, as a matter of fact, very little correlation with nutrient intakes, except as regards the source of animal proteins. Similarly, age did not appear to be a factor in the differences in intake noted between groups. As regards physical status, the

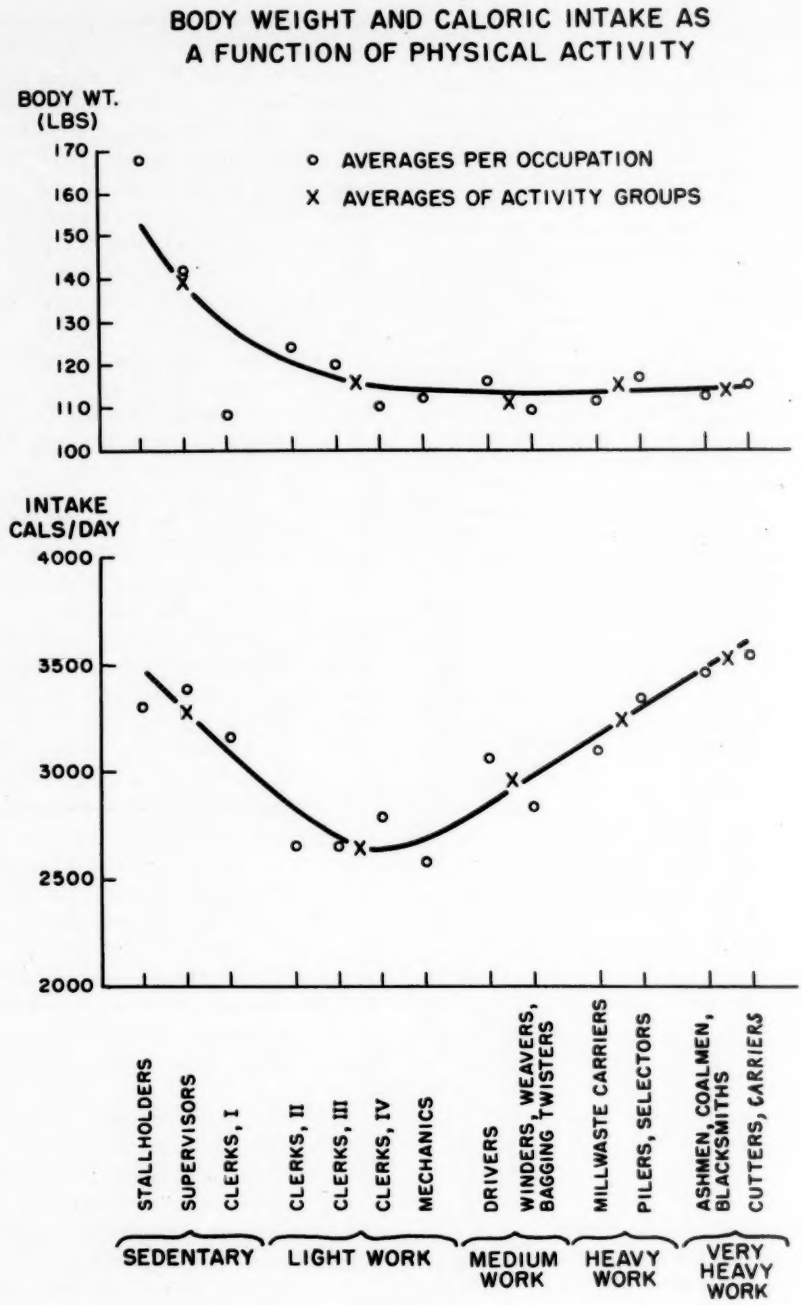
BODY WEIGHT AND CALORIC INTAKE AS A FUNCTION OF PHYSICAL ACTIVITY
BODY WT. (LBS)
170
160
150
140
130
120
110
100
o AVERAGES PER OCCUPATION
X AVERAGES OF ACTIVITY GROUPS
INTAKE CALS/DAY
4000
3500
3000
2500
2000
STALLHOLDERS
SUPERVISORS
CLERKS, I
CLERKS, II
CLERKS, III
CLERKS, IV
MECHANICS
DRIVERS
WINDERS, WEAVERS, BAGGING TWISTERS
MILLWASTE CARRIERS
PILERS, SELECTORS
ASHMEN, COALMEN, BLACKSMITHS
CUTTERS, CARRIERS
SEDENTARY
LIGHT WORK
MEDIUM WORK
HEAVY WORK
VERY HEAVY WORK

Figure 1

TABLE I

Average Daily Nutrient Intake[1] and Median of Money Spent per Week (exclusive of loan repayments and money sent home)[2]

Occupational Group	Animal Protein *g*	Vegetable Protein *g*	Fat *g*	Thiamine *μg*	Ribo-flavin *μg*	Niacin *mg*	Vit. C *mg*	Money spent per week *Rupees*
Stall holders	38.2 ± 18.7	54.6 ± 11.8	102.9 ± 44.1	1630 ± 331	1169 ± 351	14.6 ± 5.6	57.6 ± 34.3	12(8-15)
Supervisors	29.3 ± 21.2	59.7 ± 16.1	81.2 ± 36.3	2150 ± 696	1237 ± 452	14.4 ± 5.5	76.6 ± 43.3	70(28-144)
Clerks I	23.5 ± 17.7	52.5 ± 12.6	45.8 ± 35.9	1435 ± 453	848 ± 329	11.7 ± 3.7	53.6 ± 28.2	
Clerks II	17.1 ± 10.0	59.3 ± 17.5	42.5 ± 22.4	1672.4 ± 493	997 ± 391	11.6 ± 4.7	50.1 ± 21.2	50(26-168)
Clerks III	26.3 ± 16.6	56.4 ± 15.6	46.3 ± 23.6	1703 ± 517	1100 ± 436	12.0 ± 4.2	50.3 ± 11.0	
Clerks IV[3]	28.0 ± 19.9	63.1 ± 24.6	54.3 ± 27.1	1851 ± 655	1053 ± 467	13.7 ± 5.5	60.3 ± 30.7	
Mechanics	8.1 ± 7.0	53.1 ± 14.5	19.3 ± 9.7	1499 ± 366	616 ± 196	10.1 ± 2.3	37.8 ± 10.8	8(4-11)
Drivers	16.2 ± 6.8	67.6 ± 15.5	38.3 ± 16.7	1903 ± 550	904 ± 331	15.3 ± 6.5	37.7 ± 12.7	7(3-15)
Winders	5.6 ± 5.0	62.7 ± 7.2	17.3 ± 4.7	1677 ± 209	727 ± 355	11.2 ± 2.1	37.1 ± 14.3	3(2-4)
Weavers	10.3 ± 6.3	68.4 ± 15.2	23.5 ± 6.3	1829 ± 482	887 ± 219	13.5 ± 3.0	37.0 ± 5.2	10(2-18)
Bagging twisters	12.5 ± 5.7	65.9 ± 11.2	37.9 ± 16.9	1809 ± 304	779 ± 303	15.0 ± 3.3	33.3 ± 9.7	5(2-9)
Mill waste carriers	9.9 ± 3.6	71.2 ± 8.9	29.2 ± 10.3	1892 ± 199	749 ± 96	15.2 ± 2.6	29.4 ± 10.3	4(2-8)
Pilers	16.0 ± 5.4	72.2 ± 9.3	51.3 ± 9.6	1449 ± 948	973 ± 187	17.5 ± 2.5	43.6 ± 18.5	8(3-16)
Selectors	15.6 ± 7.1	77.9 ± 11.2	53.2 ± 12.0	2150 ± 697	1238 ± 452	14.4 ± 5.4	76.7 ± 43.3	6(2-12)
Ashmen, coalmen blacksmiths	12.2 ± 6.2	80.3 ± 17.1	51.6 ± 10.6	2301 ± 730	1081 ± 315	19.8 ± 8.8	32.2 ± 13.4	12(2-27)
Cutters	13.1 ± 7.7	90.1 ± 16.1	50.3 ± 15.1	2845 ± 760	1193 ± 332	25.7 ± 8.2	56.9 ± 62.2	7(1-11)
Carriers	16.2 ± 7.5	88.2 ± 11.3	58.1 ± 12.1	2108 ± 335	732 ± 187	18.9 ± 3.3	31.2 ± 9.5	6(2-17)

Figures after ± are standard deviations. One rupee = 0.21 dollar. Figures in parentheses give range.

[1] Main sources of animal protein are (in order of decreasing importance): fish, eggs, buffalo and cows' milk, meat, curds, and cheese; of vegetable proteins: rice, lentils; of fat: vegetable oil, ghee, invisible animal fat; of thiamine: rice (undermilled); of riboflavin: leafy vegetables; of niacin and vitamin C: vegetables and fruits.

[2] Difficulties are encountered when computing real income due to the communal family and living systems; thus the very low incomes reported for some workers are compatible with life because they feed themselves as members of a community of similarly situated individuals. Loan repayments have been excluded from incomes, because of the colossal interest rates (up to 30 or 40 per cent per month) often exacted by money lenders. Only medians and ranges are given for incomes; the degree of accuracy obtained, for the reasons given above, does not warrant elaborate statistical treatment. On the average, stall holders and supervisors had five dependents over the age of 10 years, three dependents in the 1–10 year range, and one half in the 0–1 year bracket. Clerks had six dependents over 10 years of age, four in the 1–10 bracket, and three-fourths under 1 year. Workers had four dependents above 10 and four under 10 (infants included).

[3] This group was the most heterogeneous in terms of physical activity because of the differences in athletic schedules between members. At least one of the men could qualify as "heavy worker" and several as "medium workers" because of their athletic activity. This variability is reflected in the larger standard deviations.

variation of weight as a function of exercise (average height 5 feet 3 inches, average weight 119 lb) is given in Figure 1. Differences in average weight between the "light work," "medium work," "heavy work," and "very heavy work" classes were not significant. The "sedentary" group was on the average much heavier. The "stall holders" and "supervisors" were obviously much fatter than the other occupation groups. Although the group "Clerks I" (very sedentary individuals) was relatively light, it may have been actually a relatively "fat" group where the visibly poor degree of muscular development obscured, from the point of view of weight, a relatively greater adiposity than in the "work" groups.

DISCUSSION

Inasmuch as it is legitimate to draw a parallel between purely experimental results and the results of a population study, there is a striking parallelism between the findings in animals[3] and those in man. In both cases it appears that food intake increases with activity only within a certain zone. By analogy with previously used terminology,[1–3] this zone can be called the "normal activity range." Below that range, in what has been termed the "sedentary" zone, a further decrease in activity is not followed by a decrease in food intake but, on the contrary, by an increase. It may be at first surprising to note that a fundamental mechanism, that of the regulation of food intake,

ceases to respond in a certain interval to variations of energy expenditure. This may be interpreted by considering the fact that, in his hundreds of thousands of years of evolution, man did not have any opportunity for sedentary life except very recently. An inactive life for man is as recent (and as "abnormal") a development as caging is for an animal. In this light, it is not surprising that some of the usual adjustment mechanisms would prove inadequate.

The increase in weight associated with inactivity appears to be of significance in relation to the problem of obesity. The importance of inactivity in various forms of experimental obesity has already been recalled. As regards human obesity, Greene[12] has reported on more than 200 overweight individuals in whom the beginning of obesity could be traced to a sudden decrease in activity. Bruch[13] has emphasized the frequency of the coexistence of physical inertia and obesity in children. Peckos[14] and Fry[15] have questioned the role of hyperphagia in the positive energy balance leading to obesity in children. Finally, in collaboration with Johnson and Burke, one of us (J.M.) has found that the average caloric intake of obese high school girls in Boston suburbs was no greater than that of the normal-weight individuals of similar age, height, and school grade, but their physical activity was very much less.[16] The fact that mechanized, urbanized modern living may well be pushing an ever greater fraction of the population into the "sedentary" range may thus be a major factor in the increased incidence of obesity.

SUMMARY

The relation between caloric intake, body weight, and physical work was established in a group of 213 mill workers in West Bengal. These workers covered a wide range of physical activity, from sedentary to very hard work. It was found that caloric intake increases with activity only within a certain zone ("normal activity"). Below that range ("sedentary zone") a decrease in activity is not followed by a decrease in food intake but, on the contrary, by an increase. Body weight is also increased in that zone. The picture is similar to that previously found in experimental animals.

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Coffee: Pro

"In January 1719, Paris became a big café. Three hundred cafés were open to conversation. It was the same in the big cities: Bordeaux, Nantes, Lyon, Marseille, etc.

"Never did France converse more or better. There is no doubt that the honour of this scintillating explosion belongs partly to the happy revolution of the times, to the great event that created new habits and modified temperaments: the advent of coffee.

"Coffee, the dark liquor, powerfully cerebral, which, altogether unlike the spiritous kind, increases clearness and lucidity; coffee which suppresses the vague and heavy poetry of the fumes of the imagination; which, out of reality, clearly seen, calls forth the spark and flash of truth. . . .

"The three ages of coffee are those of modern thought; they mark the solemn moments of the brilliant century of the mind."

—Jules Michelet. *History of France:* The Regency, 1863.

Coffee: Con

"When the Moslems drank wine the Golden Age of Eastern civilisation was in full bloom. The immortal 'Arabian Nights' was written; mathematics was at a high stage of its development; medicine and the sciences were making great progress. However, after the fifteenth century, when strong-willed fanatics got the controlling hand and prohibited the use of alcohol as a beverage, and turned the Moslems into a coffee-drinking people, civilisation began to set in the East. In fact, so stupid had the Moslem coffee-drinkers become that they had to employ alcohol-drinking Copts to keep their accounts in workable order."

—Edward Podolsky. *Med. Press* 234: 614, 1955.

Editorial

Are the Obese Metabolically Different?

The still unsolved question is: Are fat people metabolically different from those of normal weight? The final answer as applied to man is not yet on hand; however, several interesting reports have recently cast doubt on the concept that the obese have a metabolic system identical in every way with the lean.

Alonzo and Maren,[1] for example, have recently shown that while weight gain can be decreased in genetically obese mice by food restriction, the mice still had body fat concentrations two to four times as great as their lean siblings, who received more food. Under the conditions of the experiment as described, the obese mouse on an unrestricted diet had a normal total amount of protein in his carcass (total animal), but when the mouse was placed on a restricted diet, the body protein was lost in proportion to the restriction of food. In addition, these workers make this provocative statement: "Since increased activity and decreased food intake for several months did not seem to change fundamentally the fat content of the obese mice, it appears reasonable to believe that these animals metabolize their food in such a manner that more fat is deposited per gram of food intake than is the case for the lean mice."[1]

These results confirm a study on the "hypothalamic" hyperphagic rat in which, although pair-fed with *ad libitum* eating controls, these animals were found to have an increased amount of fat in their carcasses.[2] Operating here must be one or more of the "endogenous factors, as yet by no means well understood, which on occasion will enable two individuals of approximately the same size and age, having 'normal metabolic rates,' and indulging in about the same amount of daily activity, to move in different directions in regard to weight, even though their food consumptions be identical."[3]

There are, to be sure, many more questions to be asked and many more details to be filled in, but it seems to this writer that a fundamental metabolic "error" will be found in some obese persons. This disturbance will very likely be found to be an imbalance in enzyme reaction rates modified by various hormones. . . . There are, indeed, exciting days ahead.

—S. O. WAIFE, M.D.

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Eutrophy

Quite by accident I came across the word *eutrophy* which, from its Greek derivation—*eu* = well or normal and *trophe* = nourishment — means the state of good nutrition. Why the word (a cousin to atrophy) hasn't been more commonly used is, perhaps, a mystery. Certainly the term "well nourished," that is, eutrophic, is used widely enough.

At times it has been used too widely, or, at least, too loosely. For one must not forget to ask in this age of relativity—eutrophic when and where? The concepts of eutrophy held by Neanderthal man or by the Babylonians or by the Park Avenue gourmet are not similar; and even today the eutrophic male looks different in Bostonian and Bantu eyes.

It is an ancient scourge of the biological sciences that it has rarely, if ever, been possible to set "normal" standards. Countless man-hours of fruitless labor and myriads of machines and monies have only resulted in what are candidly called "arbitrary" standards. Deep within the breast of every scientist there is a voice crying out for support—support in the form of a rigid framework of "facts," from which one could say, for example, "this is hypertension" or "this is obesity." But just as Newton has been superseded by the newer physicists, so nutritional standards, epitomized in nomograms and tables, are being supplanted by the concept of "operational" or "functional" standards.

In short, we will have to change our Victorian faith in absolute quantifiable parameters and admit that eutrophy, for example, has different meanings in different settings.

It seems to me that thought should be given to what we hope to accomplish by establishing standards and definitions. For example, do we want to consider *longevity* as the star by which to steer? If so, then new studies, as well as re-evaluations of older data, will need to consider "optimum" nutrition from the point of view of its effect on the length of life. Here the experience of life insurance companies with mortality rates may come in handy, although such data are based on a highly selected population.

On the other hand, it is just as valid, philosophically, to argue that eutrophy (good nutrition) should be correlated with *health*, that is, maximum functional usefulness, happiness, and absence of disease. If so, our standards may then have to be significantly altered. (It must be remembered that after "minimum" requirements are known, there remains a largely unexplored field of "optimum" requirements. This holds true for all nutrients, from total calories to the lesser minerals.) In fact, it may be a moot point whether a short life but a merry one (full of cholesterol-laden epicurean delights) is necessarily worse than a long, lean, and lonely life of self-denial, based on chi-squared data on unappreciative mice.

This brings us to a dead end, except that the perpetual optimists will want both their cake and a long life in which to eat it. (Longevity and "health," of course, are not mutually exclusive.) But should we not reflect sometime, before we grow too old to do much about our conclusions—should we not reflect on the definition of that nice word *eutrophy*—well nourished for what?

—S. O. WAIFE, M.D.

Nutritional Enigma

"When we study Van Gogh's existence in detail, we cannot fail to be struck by his prodigious vitality. Vincent ate very badly; at Le Borinage, during a glacial winter, he hardly subsisted except by begging, nourished himself on crusts of bread or frozen potatoes picked up out of the rubbish; later, in Arles, it often happened that he lived on absolutely nothing but 23 cups of coffee for four days. One could easily multiply such examples. Although Van Gogh was constantly undernourished, he nonetheless gave himself up to the most intense work one could imagine. That he should not have succumbed fills the mind with astonishment, especially—let us emphasize this—since when Van Gogh killed himself, at the age of 37 years, he was in no way physically impaired."

—Henri Perruchot. *Revue de la Pensee Francaise* 15: 14, 1956.

Diet Therapy

Development and Role of a Food Clinic

By CLARE E. FORBES, B.S.; M.P.H.*

FROM THE latter half of the nineteenth century to the early part of the twentieth century, the Diet Kitchen Association of New York City and the South End and North End Diet Kitchens of Boston had distributed food to the "sick poor" on the basis of social and economic needs. At that time the Boston Dispensary had an affiliation with the South End Diet Kitchen. The recognition of the limitations of this practice in other fields as well as in nutrition led to the present-day trend which enables the individual to care for himself. Although the process of educating the individual is more time-consuming in the beginning, it has proved, in the overall analysis, to be less expensive and its results more lasting.[1]

One of the first to recognize the inadequacy of the practice of food distribution was the late Frances Stern, who as an instructive visiting housekeeper for the Boston Tuberculosis Association and later for the Boston Provident Association, had frequently accompanied patients on their visits to the Boston Dispensary. Miss Stern became aware of the possibilities of extending the services of the diet kitchens so that patients could be taught the wise use of food and also how their food needs might be fulfilled in view of their medical, social, and economic needs. She further felt that there was a place for a unit associated with a medical organization where patients might receive this instruction.

In this connection she sought the help of Michael M. Davis who at that time was the Director of the Boston Dispensary. His foresight and recognition of the importance of nutrition to successful medical treatment readily led to his approval and encouragement of Miss Stern's request to establish a "Food Clinic." Thus, the Food Clinic at the Boston Dispensary was established as a medical clinic service in April, 1918. As such, it was the "initial step in organized medical application of the knowledge provided by the science of nutrition."[2] Twenty-five years later the Board of Managers of the Boston Dispensary renamed the Food Clinic the "Frances Stern Food Clinic" in honor of its founder whose experience, vision, and philosophy continue to be the basis of the clinic's structure.

The original purpose of the Food Clinic was to provide direct service to the patient by interpreting the physician's food order. Essentially, this entailed teaching the patient the principles of nutrition in terms that would help him to understand the reason for and importance of adhering to the diet which had been prescribed. Inasmuch as the patient is ambulatory, he must be taught to assume the responsibility of planning, purchasing, and preparing his own diet at home. In this role, the Food Clinic continues to serve as a link between the hospital and the community.

At the Boston Dispensary the Food Clinic is professionally affiliated with and adjacent to the Medical Clinic. The facilities and services of the Food Clinic are easily accessible to patients, physicians, nurses, and social workers. The clinic consists of a large waiting room attractively furnished with round tables and chairs, pictures and objects of artistic and cultural significance which were selected for

* Chief, Frances Stern Food Clinic, The Boston Dispensary; Assistant in Medicine, Tufts University School of Medicine, Boston, Mass.

decorative and educational value. In addition, a small teaching kitchen, a nutrition workshop area, and three offices complete the food clinic unit.

In keeping with the philosophy of the Food Clinic, the nutritionist in order to function effectively must be an educator. She must realize that "motivation is at the very heart of the educational process,"[3] and that motivation results from a satisfaction of one's personal wants and goals. With this realization, the nutritionist seeks to learn the patient's immediate objectives. This knowledge in turn enables her to guide the patient who must assume the responsibility for carrying out his own diet.

The nutritional history is the means by which the nutritionist is able to define the wants and goals of the patient and teach in terms of them. During the interview, a nutritional history of the patient's past dietary habits, economic status, intellectual ability, personality, racial, religious, familial, and occupational background is elicited.[4] Since no two people have exactly the same backgrounds, wants, or goals, each diet is planned with and written for the patient individually. It is this individual planning and teaching which has continued to provide one of the most important motivating techniques used at this Clinic.

During the early years of the history of the Frances Stern Food Clinic, the majority of the patients were immigrants, and communication by word of mouth was difficult and often impossible. Therefore, in the teaching process it became essential to rely heavily on such aids as pictures, models, and actual foods, drawings or sketches, and the terms used by the foreign born. This was the beginning of the extensive use of teaching aids in the field of nutrition education. Although originally the purpose of teaching aids was to bridge the gap between the nutritionist and the foreign-born patient, it soon became recognized that these aids were an effective supplementation to educational methods for all patients.[5]

Concurrent with the problem of communication was the realization that a knowledge of the eating habits of individuals and families of various ethnic groups was necessary before the diet could be effectively planned and taught. An understanding and appreciation of the significance of food traditions and methods of food preparation for each nationality group indicated that changing the patient's native food habits to meet American standards was usually not necessary. For example, the Italian patient could meet his calcium requirement with familiar foods such as provolone, parmesan, or romano cheese. Moreover, it was easier and more effective for the nutritionist to change her teaching method.

The need for an educational method which would be universal to all nationalities was soon realized. The resolution of this problem seemed to be in the food constituent method of teaching food needs. Thus, instruction in terms of protein, fat, carbohydrate, the vitamins, and minerals rather than specific foods became a common practice. The value of teaching the food constituents has been realized in this country and is particularly appealing to visitors from foreign lands.

Through its nutrition education program, the services of the Food Clinic have been extended to the staff and patients of the Boston Dispensary and the New England Medical Center. The emphasis is on scientific nutrition information which is promoted by way of exhibits, posters, printed materials, films, and informal group teaching.

A Health Education Department, one of the first of its kind to be established in a medical institution, became affiliated with the Frances Stern Food Clinic over 30 years ago.[6] This was another phase of the nutrition education program directed to children who were considered a priority group at the Boston Dispensary. The purpose of this program was to encourage in children favorable food attitudes and practices through an understanding of the food constituents and their relationship to body needs. Nutrition was taught as it related to total health and not as an entity by itself. This was accomplished in the children's clinic by utilizing the waiting time for informal teaching.

Many lesson plans which were developed throughout the years have been compiled and

published.[7] It is interesting to note that a second generation is now participating in this program. The unsolicited evaluation from parents of children attending the clinic today, who themselves had participated in the program a generation ago, justify the program's educational value. This confirms the common knowledge that favorable nutritional practices initiated in the formative years of childhood may have potentially infinite value. This has also been one means of extending the services of the Food Clinic at the Boston Dispensary beyond the confines of the New England Medical Center to the community itself.

Although the primary purpose of the Frances Stern Food Clinic is nutrition education, both direct and indirect, a secondary function has arisen. Recognition by educational institutions of the clinic's methods has resulted in its becoming a teaching center. Students and visitors representing medicine and its allied fields, have been attracted to it from all over the world. At the present writing, over 500 students from such fields as medicine, nursing, social work, teaching, public health, and home economics, participate in the teaching program annually.

As early as 1926 dietitians and students from hospitals in and near Boston came to the Boston Dispensary for out-patient training in the Food Clinic. Ten years later, in 1936, a course of training planned to meet the specific needs of the clinic nutritionist was approved by the American Dietetic Association. The entrance requirements for the Food Clinic Internship are similar to those for the administrative and hospital internships for dietitians. However, in addition to normal and therapeutic dietetics, emphasis in the food clinic internship is placed on the educational, psychological, and socio-economic aspects of dietetics.

The Food Clinic Internship has constantly adapted its training objectives to the requirements of a postgraduate program. Thus, as the need, scope, and contributions of the internship program became recognized, academic credit at Tufts University Graduate School was granted in 1948. The intern, in addition to membership in the American Dietetic Association, can now obtain a master's degree in education by supplementing the internship with a selected academic program. The ultimate objective is to approach Dr. Lydia J. Robert's frequently quoted description of a successful clinic nutritionist:

". . .Take (a) all the knowledge of nutrition possessed by the college teacher of nutrition, (b) the special knowledge and experiences of the hospital dietitian with diet therapy, (c) the sympathy, understanding, and teaching ability of both the primary and high school teacher, (d) the knowledge of human problems and some of the techniques of the social worker and (e) the knowledge of factors influencing human behavior possessed by the psychologist. To these add: all the persuasive arts of the advertising artist, the high pressure salesman, or the stump orator, and you will have an ideal social service dietitian."[8]

This Food Clinic functions as an integral part of the Boston Dispensary and the New England Medical Center, affiliating itself closely with all departments where there is a need for joint service. It works co-operatively with community agencies in an effort to make the ambulatory patient self-sufficient and to insure continuity in the total treatment of the patient. The contribution of clinics such as this to preventive, curative, and rehabilitative medicine has been widely recognized and it is anticipated that the services of such food clinics will continue to expand with the newer developments of medicine.

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Nutrition News

Wayne Nutrition Conference

The Second Annual Nutrition Conference, sponsored by Wayne University College of Medicine, will be held in the Medical School Auditorium on Friday, April 13, beginning at 2:00 p.m. Speakers on the general subject "Nutrition and the Maintenance of Tissue Structure and Function," will include Drs. Albert G. Hogan, University of Missouri; Bertha S. Burke, Harvard University; and William J. Darby, Vanderbilt University. Further information may be obtained by writing the Department of Physiological Chemistry, Wayne University College of Medicine, Detroit 7, Mich.

International Congress of Gastroenterology

The Fifth Meeting of the International Congress of Gastroenterology will be held in London, from July 18 to 21, 1956, at the Royal College of Surgeons of England. The full program will be announced in March.

Applications for membership and requests for further information should be addressed to the Hon. Secretary, Hermon Taylor, M.D., M.Chir., F.R.C.S., at the Congress Office, The London Hospital, Whitechapel, London, E.1.

Symposium on Vitamin Metabolism

Under the sponsorship of the University of Texas and the National Vitamin Foundation, a Symposium on Vitamin Metabolism was presented on Tuesday, March 4, 1956, in New York City. Dr. Esmond E. Snell, of the University of Texas, served as Chairman, and the following papers were presented:

Some Considerations on the Metabolism of Vitamins—Dr. Severo Ochoa (New York University-Bellevue Medical Center, New York)

The Metabolism of Thiamin and Lipoic Acid—Dr. I. C. Gunsalus (University of Illinois, Urbana)

The Metabolism of Riboflavin—Dr. G. W. E. Plaut (New York University-Bellevue Medical Center, New York)

The Metabolism of Niacin—Dr. L. M. Henderson (University of Illinois, Urbana)

The Metabolism of Vitamin B_6—Dr. Jerard Hurwitz (National Institutes of Health, Bethesda)

The Metabolism of Pantothenic Acid—Dr. G. M. Brown (Massachusetts Institute of Technology, Cambridge)

The Metabolism of Folic and Folinic Acids—Dr. C. A. Nichol (Yale University, New Haven)

The Metabolism of Ascorbic Acid—Dr. J. J. Burns (New York University, New York and National Institutes of Health, Bethesda)

The Metabolism of Biotin—Dr. Lemuel D. Wright (Sharpe & Dohme, Division of Merck & Co., Inc., West Point, Pa.)

U. S. Army Medical Nutrition Laboratory

The U. S. Army Medical Nutrition Laboratory in co-operation with the Quartermaster Research and Development Command, Natick, Mass., has been conducting a controlled metabolic balance study on a platoon of 26 soldiers in a bivouac situation during the winter of 1956, at Fort Churchill, Manitoba, Canada. The subjects have volunteered for the duty. The object is to determine the caloric and nutrient requirements of human subjects and the physiologic and biochemical changes which occur in men who are exposed to a situation of extreme cold and great physical exertion for an extended period of time.

The Army Medical Nutrition Laboratory is a research installation under the Research and Development Division, Office of the Surgeon General. It was established in 1944 in Chicago, Ill., and it was moved in 1953 to the grounds of Fitzsimmons Army Hospital, Denver, Colo. It has a staff of about 100 personnel, of whom 75 are technically trained. During the fiscal year 1955, the laboratory had 58 publications and the staff presented more than 100 papers and lectures before scientific groups throughout the country. The installation is directed by Lt. Colonel L. M. Hursh, M.C., Commanding Officer; Lt. Colonel Carl J. Koehn, M.S.C., Deputy Commanding Officer; and Theodore E. Friedemann, Scientific Director.

Nutrition Briefs

CURRENT OBSERVATIONS OF CLINICAL INTEREST

LYSINE deficiency causes retardation of bone growth, but no interference with bone healing. Other amino acid deficiencies similarly retard growth.

R. Haggs, T. D. Kinney, and N. Kaufman. *J. Nutrition* 57: 305, 1955.

NOT ONLY is severe atherosclerosis rare among adult Bantus, but fatty liver is rare among Bantu alcoholics. It is possible that pancreatic elastase (which apparently affects elastic tissue and is also lipotropic) may be unusually active in such persons. Research among certain primitive groups is essential.

A. R. P. Walker and M. Andersson. *Lancet* 2: 1300, 1955.

ADMINISTRATION of β-sitosterol to men under age 45 who had recovered from myocardial infarction resulted in a significant fall in serum cholesterol, total lipid, and β-lipoprotein lipid. No difference in response to sitosterol was noted between patients on a regular and on a low fat, low cholesterol diet.

J. W. Farguhar and R. E. Smith. *Clin. Res. Proc.* 4: 55, 1956.

DECREASED fat absorption may be important in the weight loss following subtotal gastric resection. There is an inverse relationship between the efficiency of fat absorption and the amount of stomach removed. Those patients who tolerate oral fat emulsions often show an improvement in fat absorption.

E. H. Ellison. *Surg. Clin. N. Amer.* 35: 1683, 1955.

METABOLIC studies in a 37-year-old man indicate that compound F (hydrocortisone) accelerates fat deposition, although compound A (11-dehydrocorticosterone) has no such effect.

L. H. Kyle, R. L. Meyer, M. Schaaf, E. J. Werdein, and J. J. Canary. *Clin. Res. Proc.* 4: 16, 1956.

CAFFEINE AGAIN: Reporting an error in a previous analysis of the caffeine content of various coffees, the A. M. A. now indicates that "the available decaffeinated ground lean coffees contain roughly one-fortieth of the amount of caffeine found in regular coffee." Decaffeinated instant coffee is similar. This is equivalent to 97 per cent or more removal of caffeine.

J. A. M. A. 159: 250, 1955.

RECENT ADVANCES IN EXPERIMENTAL NUTRITION

HIGH doses of chloramphenicol in dogs are associated with severe inanition, marrow hypocellularity, and high mortality. Similar findings can occur after other antibiotics if food intake is similarly restricted. The reported abnormalities after experimental chloramphenicol administration are malnutritional effects secondary to anorexia produced by large doses of the drug.

J. L. Radomski, A. A. Nelson, and B. W. Deichmann. *Antibiot. & Chemother.* 5: 674, 1955.

MICE rendered deficient in riboflavin, pyridoxine, and pantothenic acid show a greater tissue susceptibility to tobacco smoke than do adequately nourished animals.

S. J. Kreshover. *J. Dent. Research* 34: 798, 1955.

FOOD consumption may be correlated with hours of daylight in certain animals. Studies on adult lizards reveal seasonal changes in body composition not due to temperature differences.

H. C. Dessauer. *Proc. Soc. Exper. Biol. & Med.* 90: 524, 1955.

TWO unidentified growth factors for chicks have been described. They are present in liver: one is organic, the other probably inorganic. (Is a new vitamin soon to be isolated?)

H. M. Edwards, Jr., D. Chin, L. C. Norris, and G. F. Heuser. *J. Nutrition* 57: 229, 1955.

THE resistance of mice to experimental tuberculosis was increased by feeding a group of fatty acid esters (found in coconut oil). Lard or methyl linoleate did not have this effect.

L. W. Hedgecock. *J. Bacteriology* 70: 415, 1955.

THE MOST potent known antagonist to nicotinamide is 6-aminonicotinamide. Its toxic effects apparently are produced by depleting some tissues of DPN (diphosphopyridine nucleotide). Tryptophan appears to give some protection.

W. I. Johnson and J. D. McColl. *Science* 122: 834, 1955.

MOST of the uncinate process of dog pancreas is devoid of alpha cells in the islands of Langerhans, whereas they are found in the remainder. No hyperglycemic activity was found in pancreatic extracts from the uncinate region. Results suggest that glucagon originates from alpha cells.

S. A. Bencosme, E. Lieper, and S. S. Lazarus. *Proc. Soc. Exper. Biol. & Med.* 90: 387, 1955.

Nutritional Quotes

Recommendations and Restraint

"Apart from frank deficiency states which call for medical advice and presumable therapeutic treatment, the levels of vitamins and minerals supplied as additives should furnish a substantial part of those represented by the Recommended Dietary Allowances, but should not exceed them. To argue that higher levels should be used is to argue that the allowances are wrong by being too low for their established purpose of maintaining good nutrition in healthy persons, or that the pure nutrients are less well utilized than those in natural foods."

—L. A. Maynard. *Journal of the American Dietetic Association* 31: 331, 1955.

"Supplements" vs. a Good Diet

"Many patients in a hospital for chronic diseases had been taking large doses of brewer's yeast over long periods of time—months and years. The utter inadequacy of this therapeutic approach was quickly revealed by a mere glance at the large number of cachectic persons in this group. In other words, vitamins per se, or any other preparation designed to stimulate eating, are totally ineffective without provision of a diet containing sufficient calories. We believe that the treatment for undernutrition is a good diet, not vitamin supplements."

—J. I. Goodman and W. Dowdell. *Ann. Int. Med.* 43: 1256, 1955.

Hereditary Susceptibility to Rickets?

"As is so often the case, the work I am about to describe was begun for quite another purpose. We wanted to study the excretion of amino-acids in the urine of children who were fed with human milk and cow's milk. We then observed that some of our children, especially some premature babies, began to excrete an increased amount of amino-acids when they were some months old. It became apparent that these infants had not received any vitamin D and that they showed the first signs of rickets. When vitamin D was added to their diet the amino-acid excretion went back to normal. We believed it worth while to study the possible relation between rickets and amino-acid excretion. In a number of rachitic children we determined the amino-acid excretion and did the same after the children had been cured. In all patients with active rickets the amino-acid excretion was found to be increased, especially in the severe cases. In most cases the increased amino-acid excretion was twice or three times the normal value. This increase was mainly due to an increase in the excretion of free amino-acids. The excretion of amino-acids in the form of peptides or conjugates rose only slightly. In all cases the amino-acid excretion diminished after vitamin D had been given, but not always to normal values. We found that in those children in whom, after the healing of rickets, the amino-acid excretion remained somewhat high, the rickets had a more severe course and that more vitamin D than normal had been necessary in these cases. In some of these, other children from the same family showed signs of having had rather severe rickets. The amino-acid excretion was found to be higher than normal in these children also, although their rickets was no longer active. We could observe this peculiarity in various families. It looks as if the increased susceptibility to rickets and the need for more vitamin D than is normally necessary go together with an increased excretion of amino-acids in the urine. We think that both peculiarities are hereditary and concomitant."

—J. H. P. Jonxis. *Glasgow M. J.* 36: 230–232, 1955.

Atherogenic Diets and Protective Factors

"With respect to dietary factors influencing response to a cholesterol-containing, potentially atherogenic diet, it may be briefly stated that significant changes in hypercholesterolemic and atherogenic response of chicks were effected by chronic undernutrition, withdrawal of neutral fat, addition of excessive neutral fat, supplementation with plant sterols or a defatted brain extract. In contrast, numerous so-called lipotropic factors—choline, inositol, pancreatin, activated whole pancreas, anti-fatty liver factor, tocopherols, vitamin B_{12}, lipocaic, etc.—were without influence on hypercholesterolemia and atherosclerosis in cholesterol-fed intact or depancreatized chicks."

—Louis N. Katz. *Ann. Int. Med.* 43: 935, 1955.

Inadequate Protein and Atherosclerosis

"With the monkey, a primate species like ourselves, we have been successful in producing atherosclerosis by dietary means. Inadequate protein and high fat diets are necessary for its production.

. . . "The contribution that our laboratory may have made is the discovery that in the primate poor protein in the diet is necessary in addition to increased amounts of fat and cholesterol in order to produce atherosclerosis. By 'poor' protein is meant an insufficient amount of the sulfur amino acids, methionine and cystine.

"This will no doubt raise the question of whether or not the methyl group of methionine is the material concerned. I think definitely that it is not. Choline, a substance necessary as a methylating agent in the body, must be present in sufficient amounts if atherosclerosis is to occur. From our experimental studies, it would appear that organic sulfur is the protein component involved in the development of atherosclerosis."

—F. J. Stare. *J. Am. Geriatric Soc.* 3: 769, 1955.

Fasting and Infection

"The infection-enhancing effect of fasting becomes manifest within thirty-six hours and can be rapidly corrected by feeding a complete natural diet *ad lib.* In contrast, it is further aggravated when glucose, or an incomplete diet, is given to the animal during the fasting period. It could not be corrected by feeding a diet containing sugar, fat, minerals, all known vitamins, and all essential amino acids, for five days before challenge with living bacteria or toxin."
—René J. Dubos, J. Maclean Smith, and Russell W. Schaedler. *Proc. Roy. Soc. Med.* 48: 914, 1955.

Vitamins in Infectious Diseases

"The influence of vitamin nutrition on resistance to specific infections in the human subject remains largely unknown. There is considerable evidence that infections, and other stress situations, influence the requirement or utilization of certain vitamins, but there are few, if any, data regarding exact requirements in specific pathologic states. The therapeutic value of administration of large doses of vitamins in infections has not been appraised critically."
—G. A. Goldsmith. *New England J. Med.* 254: 164, 1956.

Prerequisite for Atherosclerosis

"The ingestion over the years of a diet rich in cholesterol-lipid is an *essential prerequisite* for the development of significant atherosclerosis in a population group. Such a diet may be regarded as an essential 'trigger' for the atherogenic process (viewed as a group phenomenon). Once the trigger is pulled (i.e., once the prerequisite diet is ingested), individual differences—endogenous factors (hereditary, constitutional, etc.)—come into play to influence whether the charge is fired (i.e., whether the given person develops significant atherosclerosis)."
—Louis N. Katz. *Ann. Int. Med.* 43: 933, 1955.

Digestion in Celiac Disease

"If we examine the available information as to the processes of digestion in the children with celiac disease we find several abnormalities in spite of the lack of anatomic change. Duodenal amylase derived from the pancreas is absent or present in subnormal amounts. This is the probable explanation for the clinical intolerance to starch and the presence of excess starch in the feces. However, normal amounts of salivary amylase are present, which is perhaps why these children can tolerate Melba toast or zwieback better than cereal—they are more likely to apply the saliva to the toast. In many cases, the gastric juice is deficient in amount, in acid, in capacity to form curd, and in pepsin. The lack of rennet action is a possible reason for their intolerance to whole milk, while junket, cottage cheese, and protein milk are well tolerated. Patients who lack acid tend to have poor digestion of vegetable fibers, and more vegetable particles are visible in their feces."
—Dorothy H. Andersen and Emma M. Mike. *J. Am. Dietet. A.* 31: 343, 1955

Vitamin B_6 Balance Sheet

"The daily requirement of vitamin B_6 for human beings is estimated to be 1 to 2 mg.; the average American diet provides this much with ease. The average amount of vitamin B_6 lost in the stool is 0.5 to 0.8 mg. The average amount lost in the urine as the active vitamin is 0.2 to 0.3 mg. and as pyridoxic acid 3 to 4 mg. In order to bring these data into balance, one must remember that the vitamin lost in the stool has been derived primarily from micro-organisms and does not represent food vitamin B_6 and that the pyridoxic acid values are erroneously high. It is probable that a balance occurs at an intake-output of about 2 mg. Very little vitamin B_6 is found in the blood. Serum levels average about 5 mcg. per 100 cc. Pyridoxal phosphate levels are even lower. Tissue levels have not been determined often enough in human beings for a statement of values, though the level for normal skin appears to be about 1 mcg. per gram (dry weight.)"
—Richard W. Vilter. *J. A. M. A.* 159: 1210, 1955.

Diet in Cirrhosis

"The fact that protein deficiency may be an important etiological factor in cirrhosis does not imply that a high rather than a normal protein diet is necessary as treatment. In fact, most clinics now emphasize that it is far more important that the patients eat food of adequate quantity and quality than it is that a specific or peculiar diet be furnished."
—C. S. Davidson. *J.A.M.A.* 160: 391, 1956.

Disability and Diet

"The great prevalence of malnutrition among chronically disabled persons, as revealed by this study in a country so well stocked with food as the United States, is frustrating and irksome, if not actually shocking. The importance of the present observations is further accentuated by recent study showing that malnutrition per se is the sixth highest cause of prolonged disability. In other words, these malnourished individuals could have remained well and normally active if they had not failed to consume an adequate diet. Undernutrition, with its various manifestations, is not a disease exclusive of the chronically ill and the aged, but occurs in the so-called normal population as well. Actually, it will be pointed out that the incidence of undernutrition and underweight among so-called 'healthy' individuals (33%) is almost as great as the incidence of this condition among sick people."
—J. I. Goodman and W. Dowdell. *Ann. Int. Med.* 43: 1253, 1955.

Reviews of Recent Books

Modern Nutrition in Health and Disease, edited by M. G. Wohl and R. S. Goodhart, Lea and Febiger, Philadelphia, 1955, pp. 1062, illust. 207, $18.50.

This text is a revised version of the popular volume *Dietotherapy* edited by Wohl 11 years ago. Those familiar with the earlier work will recognize that extensive reorganization and rewriting of the material has taken place. This is an obvious necessity because of the rapid advances which have evolved within the past decade in the science of nutrition. In order to achieve the objective of presenting "the salient advances in nutrition that have a direct bearing on the maintenance of optimum health and the prevention and treatment of the disease," the editors have assembled in this volume the contributions of 55 eminent authorities in this field.

Among the important additional discussions included in the present volume are those on body composition by Keys, hormonal control of metabolism by Ralli and Dumm, and the physiology of hunger by Brobeck, as well as other topics reflecting the newer knowledge accumulating from current nutritional studies. The book is arranged in three parts, the first of which deals with normal nutrition. The chapters devoted to protein metabolism by Geiger, and fat metabolism by Deuel, are excellent in their presentations of fundamental developments within these spheres of interest. The discussion of the vitamins is concisely abbreviated. The relatively small space devoted to many of the B-complex group is compensated by a chapter on the relation of diet to oxidation enzymes. The discussion of dietary interrelationships by Sure is admirably complete, being derived from an extensive list of references. Part 2 of the text is concerned with nutrition in disease. In this section, one finds an outstanding, well-illustrated dissertation on nutrition in relation to Dental Medicine by Shaw. In addition, there are chapters presenting the nutritional aspects of gastrointestinal diseases, diabetes, allergy, liver diseases, renal disorders, skin, nervous system diseases, and others in which diet therapy is an essential element. The emphasis on the practical aspects of dietary management is much in evidence. Part 3 deals with nutrition in periods of physiologic stress and contains an important treatise on food and nutrition in relation to work and environmental stress by Shils. Other physiologic stresses considered in the final section are geriatrics, pediatrics, pregnancy, and emergency feeding problems, each prepared by workers who have pioneered in these respective fields.

This book, through the completeness of its coverage of nutritional aspects of medicine, its subspecialties and related subjects, is destined to be one of the most important reference texts for physicians and students available at the present time. The relatively brief discussion devoted to some of the vitamins and deficiency diseases is adequate for practical purposes; biographic references are provided for more thorough study of these subjects. This text can be unreservedly recommended as essential reading for all physicians engaged in the treatment of disease and for students who, in general, receive inadequate training in nutrition.

C. R. SHUMAN

Mental Hygiene in Public Health (second edition), by Paul V. Lemkau, McGraw-Hill Book Co., Inc., New York, 1955, pp. 486, $8.00.

This very well written, interesting and authoritative text is probably the leading resource book available on the subject of public mental health. The author's general approach to the extremely far-reaching subject matter of mental hygiene as an area of public health planning is eclectic and not bound rigidly to any particular school of psychiatric thinking. Some may regard it as slightly unfortunate that the author has perhaps given too little attention to Dr. Freud's significant contributions to the understanding of personality development.

The scope of mental hygiene is so great that a book of a mere 500 pages can hardly be expected to be comprehensive. Nevertheless, Dr. Lemkau succeeds in touching upon most of the essentials in public mental hygiene programs. A sophisticated work, the book is nonetheless readily comprehended and is replete with illustrative case material well chosen for its applicability to everyday life. Hardly any student or professional in the field of public health or public welfare will fail to find something to interest him in this delightful text.

From the point of view of the nutritionist, there are several references in the book to the areas of relationship between nutrition and emotional disorder. Feeding and feeding problems of infancy, as well as the nutritional aspects of certain psychoses, are given brief consideration. It is perhaps slightly regrettable that the author has not allocated a greater quantity of content to nutrition's place in the field of mental health, because there is immanent in the field of nutrition a vast realistic function in preventive psychiatry. The student in this field would certainly desire a more extensive consideration of such topics as infant-parent relationships intrinsic in the feeding operation; the significance of nutrition in such psychoses as delirium

tremens, Korsakoff's psychosis, certain toxic psychoses, and pellagra; and the very important place played by nutrition in the emotional problems of late age. It would also be desirable were there a more complete discussion of malnutrition as a form of social deprivation leading to emotional difficulties, as well as a consideration of the important role that nutrition plays in the therapy and rehabilitation of mental disturbances.

The author is a pioneer in the field of public mental health. Recent progress in the development of mental hygiene and the nationwide increased interest in this social pursuit are admirably reflected in Dr. Lemkau's revised and current material. Not only will a reading of this work serve as a stimulating introduction to the understanding of human nature, but, in addition, the information made available in the several appendices will enable the student to broaden his fields of knowledge by utilizing the extensive lists of references.

The tone of the text is simultaneously sound and humble. The author does not attempt to record the accumulated body of facts in mental health without frequently calling the reader's attention to what is not known. It thus serves also as a guidepost for research.

This is an altogether rewarding book for individuals who are interested in their own and social welfare.

MAURICE E. LINDEN

Transactions of the 4th Meeting of the International Society of Geographical Pathology, edited by H. Betz and F. C. Roulet, S. Karger, Basel and New York, 1953, pp. 652, Swiss fr. 46.80.

Our review of these transactions is regrettably late, and much work has been devoted to the subject of the meeting—hepatitis—since their publication. However, those who are not daunted by trilingual presentation will find here a great deal of valuable and by no means out-dated material on the various hepatitides. The descriptions of the peculiar features of hepatitis as it is seen in a number of countries and in a variety of pathologic states are especially interesting. There are numerous discussions of clinical and pathologic pictures, epidemiologic studies, and reports on biochemical findings and on animal experimentation in several species. The relationships between infectious hepatitis and cirrhosis, between hepatitis and cancer, and the influence of hepatitis on diabetes and pregnancy are explored.

Of special interest to nutritionists is Glynn's paper on "Nutritional Factors in the Aetiology of Hepatic Diseases" (in English), and Voigt discusses "The Dysproteinemia of Virus Hepatitis" (in German).

The coverage is world-wide, both with respect to subject matter and contributors. English readers will particularly appreciate the excellent papers by McCallum and Sherlock.

A.E.S.

Advances in Food Research, Vol. VI, edited by E. M. Mrak and G. F. Stewart, Academic Press, New York, 1955, pp. 398, $9.00.

The following are a few of the salient points covered in this 398-page volume, in which results of the researches of seven food industries have been thoroughly reviewed by specialists in the fields.

Important properties for candy-making applications have been determined in considerable detail for fats and starches. Significant recent progress has been made in stabilizing animal fats, particularly butter, against oxidative rancidity. Cocoa and chocolate have been studied more extensively for their specific applications in candy making than have any of the other ingredients of candies. The review on the spoilage of wines covers a literature of 100 years in a critical manner and interprets existing concepts in the light of recent knowledge. In the chapter on microbiology of animals it is stressed that, where practical, ultraviolet irradiation should be employed to reduce surface contamination and to permit more rapid tenderization. Use of antibiotics should be evaluated by the meat industry as a means of reducing contamination on meat surfaces. Most spoilage encountered in the frozen food field is due to improper handling of the product either prior to freezing or after it is thawed. In studies of the thermal destruction of thiamine in foods, the value of the kinetic approach has become evident. If further work is done along that line, one may expect rapid solutions of problems still outstanding.

This volume of *Advances in Food Research* should prove of immense value to all food technologists and industrial engineers engaged in food industries.

B. SURE

The Role of Algae and Plankton in Medicine, by Morton Schwimmer and David Schwimmer, Grune and Stratton, 1955, pp. 85, $3.75.

During the past several decades numerous articles have appeared by botanists, nutritionists, and other scientific writers on algae and planktons as possible sources of foods for the increasing world population. This monograph is the first to assemble such knowledge of algae as is of interest to medicine. The microscopic algae are the simplest form of plant life, and the macroscopic algae are the seaweeds, which are used considerably in the Orient as extenders of foods, i.e., in soups, noodles, and in sauces. The planktons are divided into the phytoplanktons, which are the microscopic algae, and the zooplanktons, which are microscopic animals consisting of fish eggs and larvae of crabs, molluscs, and copepods.

The Chinese were perhaps the first to use algae for various diseases, especially goiter, which is understandable because of their high iodine content. The value of agar as a laxative is recommended by the U. S. Dispensatory because of its colloidal property of absorbing and holding water without being digested. Since its utilization by Koch in 1881 for the culture and isolation of pathogenic organisms, agar has become an important adjunct of every hospital and bacteriological research laboratory. It is also used as a sealer and coating for pills.

Alginic acid is the hydrophilic colloidal polymer of

anhydro-beta-D-mannuronic acid, which is extracted from brown algae, especially the giant kelp. Alginates are being used in medicine as follows: (1) as a wound dressing; (2) as a mold for applying skin grafts; (3) as a hemostatic in brain and thoracic surgery; (4) as a decontaminant and therapeutic agent in mustard gas poisoning and burns; (5) as swabs in the bacteriological examination of eating utensils, since alginate "wools" adsorb more bacteria on wiping than does cotton. Anticoagulant and antibiotic properties of algal substances have also received attention. Laminarin, a product obtained from algae, has been used successfully as a blood plasma substitute on dogs in shock.

Microscopic algae have also been assigned an indirect role in some human disease states. The most familiar intoxications are the acute "paralytic" shellfish poisoning resulting from eating mussels and clams.

This is the most complete treatise on the use of algae in medicine. The word *plankton* in the title is superfluous, because only the phytoplanktons, which are microscopic algae, have a role in medicine. B. SURE

Les Gastrites, by F. Moutier and A. Cornet, Masson, Paris, 1955, pp. 404, bound 4.400 fr., paper 3.800 fr.

The problems raised by this study of gastric disorders are important ones, and their relevance is not limited to purely gastric questions. They also touch on complex biologic dysfunctions which involve, in the course of their development, almost the entire organism. The authors have approached this formidable subject armed with the experience of more than 12,000 gastroscopies. They attack it *qua* internists, familiar with general physiology and with all the techniques of medical science. For many problems they can offer conclusions, and they have also endeavoured to be as complete as possible, for, without neglecting older studies, they have given the greatest weight to the modern research orientation.

A discussion of the chemistry and cytology of the stomach precedes a radiologic description applied to the various gastric entities, followed by a study of the normal and pathologic histology of the stomach wall, as well as of the evolutionary processes of its changes. The role of biliary reflux, clearly demonstrated by Lambling, is here given its due place. Radiology retains an honorable but restricted place in the diagnosis of gastric conditions.

The authors make a particularly careful and abundantly illustrated study of the histology of the gastritides. The reader can follow with them the elementary processes which lead, in a number of cases, to a single and common outcome: atrophic gastritis.

The clinical chapters are approached from the point of view of the problems posed by different etiologic factors. It is well-nigh impossible to set up simple schemata, and the authors recognize this very frankly. The "superacute" gastritides, rare though they may be, are the object of a detailed analysis. Following this, the chapter on acute and subacute gastritides takes into account the different types of ulcerous gastritis. This allows one to understand the complex development of hypertrophic and atrophic chronic gastritides. Ingenious symptomatic and anatomic condensations permit the investigator to analyze and classify his personal cases.

Also studied are the gastritides seen after partial gastrectomy, with cutaneous disorders, and with diseases of the blood. The role of allergy in the gastritides is also discussed. This role is probably quite important but is often difficult to prove in the absence of any foolproof objective criterion.

What about the relations between ulcer and gastritis? Cruveilher's ulcer is not derived from a gastric ulceration. The two histologic entities are different. But the ulcer is often accompanied by a gastritis of one type or another. Cancer and gastritis: a difficult problem, about which one can say that every chronic gastritis creates a terrain favorable to the development of cancer, without necessarily determining its developmental potential.

Therapy occupies a prominent place in this work, whether it is a question of the treatment of gastritides with microbial infection by antibiotics, of antigastralgics, of synthetic antihistamines, or of anticholinergic agents. The role of surgery in the gastritides is restricted and exceptional. The authors trace all the nuances of its indications, possibilities, and dangers.

The bibliography comprises 600 references, most of them to recent works. This quite remarkable work, written with a typically French clarity, marks both a point of arrival and a point of departure, and represents an essential contribution to both gastropathology and general pathology. HUGUES GOUNELLE

Books received for review by the AMERICAN JOURNAL OF CLINICAL NUTRITION are acknowledged in this column. As far as practicable those of special interest are selected, as space permits, for a more extensive review.

The Diabetic's Cookbook, by C. B. Strachan, Univ. of Texas Press, Austin, 1956, pp. 304, $6.50.

Inorganic Nitrogen Metabolism: Function of Metallo-Flavoproteins, edited by W. D. McElroy and B. Glass, Johns Hopkins Univ. Press, Baltimore, 1956, pp. 728, $10.00.

Protein Malnutrition—Proceedings of a Conference in Jamaica, edited by J. C. Waterlow, Cambridge Univ. Press, 1955, pp. 277.

Ciba Foundation Symposium on Experimental Tuberculosis: Bacillus and Host with an Addendum on Leprosy, Little, Brown & Co., 1955, pp. 396, $9.00.

Abstracts of Current Literature

CHARLES R. SHUMAN, M.D., EDITOR

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SPECIAL ASPECTS OF PROTEIN NUTRITION

The recommendation that dietary protein be restricted in patients with renal disease has not received acceptance in all quarters. Studies have been presented in the past showing that in acute nephritis, as in the normal subject, a high protein intake may be beneficial to renal function: the morbidity and duration of the disease were reduced in the protein-treated group as compared to the low protein group. In the presence of azotemia, however, there are cogent reasons for reduction of protein intake.

The Influence of Dietary Protein Intake on Specific Renal Functions in Normal Man. T. N. Pullman, A. S. Alving, R. J. Dern, and M. Landowne. *J. Lab. & Clin. Med.* 44: 320, 1954.

Clearances of inulin, diodrast, para-aminohippurate, and "maximum tubular secretory capacity" of para-aminohippurate were ascertained in 20 young, healthy adults. These measurements were made in each of the subjects after two weeks of one of three dietary regimens: Protein intake of 2.3–3.0 g per kg per day: 1.0–1.4 g per kg per day; 0.1–0.4 g per kg per day. Although these diets were kept isocaloric and sodium chloride intakes were approximately equated, intakes of electrolytes in general and of other factors were evidently not rigidly controlled. The above designated renal functional assessments tended to be augmented by the higher protein intakes and to be depressed by reduced intake of protein. Excellent experimental design characterizes this contribution to a controversial field.—R. TARAIL

Influence of Protein Diets on Development of Renoprival Hypertension in Dogs. W. J. Kolff and I. H. Page. *Am. J. Physiol.* 181: 580, 1955.

These experiments demonstrated that the hypertension resulting from nephrectomy is accentuated by overhydration with an electrolyte solution resembling extracellular fluid. However, intermittent peritoneal lavage did not prevent the production of hypertension. High protein diets containing 4–6 grams per kilogram of casein per day, with or without salt, were fed to dogs and compared to fasted controls. The high protein diets produced a minimal increase in renoprival hypertension. Medial arteriolar necroses were more frequent and more severe in those fed high protein diets. The same statement can be made for focal myocardial necroses.—M. J. OPPENHEIMER

The peptide linkages of the amino groups in protein molecules are hydrolyzed by the gastrointestinal proteinases. Pepsin, the gastric proteolytic factor, is capable of splitting proteins into their component groups, including free amino acids and peptides.

Canine and Human Gastric Digestion of Proteins in Vivo. E. S. Nasset and A. Davenport. *J. Appl. Physiol.* 7: 447, 1954.

When gastric juice was collected from dogs, at least 15 microbiologically available amino acids were present. Precautions were taken to prevent duodenal regurgitation. These amino acids were present when egg albumin or zein was fed. However, they were also found when the dog was induced to secrete saliva and gastric juice by the sight and smell of appetizing food. Although lysine and tryptophan are absent from zein, they were always found in gastric contents, no matter what protein test meal was used. The authors make the point that a rather complete mixture of amino acids is presented to the duodenum from the stomach. Part of these are secreted as free acids. Hydrolysis of ingested protein, as well as enzymes and proteins present

in saliva and gastric juice, also furnish amino acids.—M. J. OPPENHEIMER

The lipotropic effect of high protein feedings reported below is similar to that observed with administration of pancreatic trypsin to depancreatized animals with fatty liver. In the latter study, the anti-fatty liver factor of pancreas proved to be the proteolytic enzyme which restored protein digestion.

The Lipotrophic Activity of Protein. C. C. Lucas and J. H. Ridout. *Canad. J. Biochem & Physiol.* 33: 25, 1955.

In experiments carried out on groups of male rats, more fat was found in the livers of those on diets low in protein than in those on more adequate rations, in spite of the same amount of choline, methionine, and vitamin B_{12} in all diets. It therefore seems that protein exerts a lipotropic effect which is mediated by some process not directly involving choline or any of its known precursors.

It is suggested that protein contributes to the protection of the liver in at least three ways: (1) by supplying methionine which prevents accumulation of excessive fat in the centrolobular regions; (2) by exerting a lipotropic effect which is independent of the methionine content and which seems to act specifically in the periportal regions; (3) by providing the building blocks necessary for the maintenance and repair of liver cells.

The distribution of fat in the livers of rats fed low-protein diets is periportal, similar in type to the fatty liver of kwashiorkor.—F. E. HYTTEN

Changes in Pancreatic Enzymes Brought About by Alteration in the Nature of the Dietary Protein. D. F. Magee, and E. G. Anderson. *Am. J. Physiol.* 181: 79, 1955.

When animals were maintained on a diet high in casein the resting pancreas contained more trypsin and lipase than those from controls which had received gelatin, zein, or glucose. This throws doubt on previously reported experiments in which a decrease in fecal fat was observed after zein or gelatin was fed and from which an increase in pancreatic lipase was postulated. Urecholine increases pancreatic lipase and trypsin in the resting organ. If the diet is supplemented with *dl*-valine, similar results were obtained. The authors consider valine as a potent factor in the release of pancreozymin. They are of the opinion that an adequate stimulus for pancreozymin is more important than the nutritional sufficiency of the protein used in production of lipase and trypsin.—M. J. OPPENHEIMER

In the following report, retention of nitrogen is observed in underweight patients with hepatitis. This is similar to the earlier observations reported of a lesser degree of nitrogen loss following stress in nutritionally impoverished patients.

Studies on Nitrogen Balance in Acute Viral Hepatitis. I. C. Plough, B. T. Forsyth, P. E. Teschan, and V. M Sborov. *Am. J. Med. Sc.* 230: 182, 1955.

Protein-rich diets have been beneficial in the treatment of chronic liver disease and have resulted in improvement in patients with acute viral hepatitis. Evidence was sought of changes in nitrogen balance with improvement in liver function of five patients with hepatitis under treatment in a metabolic unit. Two of the patients who were underweight and, perhaps, nutritionally depleted, retained nitrogen throughout the period of study. Nitrogen losses occurred in two other patients where the study periods represented a decrease in protein and caloric intake. They, however, were in nitrogen balance toward the end of the study. The final patient experienced a marked loss of nitrogen during the administration of ACTH used in the treatment of hepatitis. The results of this study provide evidence that patients with viral hepatitis can retain ingested nitrogen as well as those without hepatitis. Because high protein feedings apparently shorten the course of hepatitis, and the present study suggests that the efficiency of protein utilization is normal, the therapeutic effect of dietary protein would seem to be related to restoration of an existing deficit of protein.—C. R. SHUMAN

THINNESS AND UNDERNUTRITION

The thin, "underweight" individual may be entirely normal although his body weight is considerably below that recommended in the standard weight tables for height and age. The body compartment measurements in these persons disclose a low content of fat with normal lean body mass and normal or low body water. In contrast, the undernourished patient exhibits low fat and low lean body mass with an increased content of water. The thin normal person may be thin because of increased physical activity, so that excess calories are rapidly disposed of through his continual muscular activity and random movements. The periodic measurement of energy expenditure at four-hour intervals may not detect this type of caloric utilization.

Energy Utilization in Overfed Thin Young Men. R. Passmore, A. P. Meiklejohn, A. D. Dewar, and R. K. Thow. *Brit. J. Nutrition* 9: 20, 1955.

Some people keep in good health, appear to have a good appetite, and yet remain very thin. Yet no physiologic abnormality has been demonstrated in such people.

Three constitutionally thin young men were fed on diets from 1300 to 1600 cal in excess of requirements for periods of 10 to 14 days. During this time they followed a standard regime of physical activities while resident on the metabolic ward of a hospital. Energy expenditure was determined by indirect calorimetry at 4-hourly intervals throughout 24-hour periods. Measurements showed that the excess food was effectively absorbed, but no more was oxidized than

could be accounted for by the specific dynamic action of the extra protein consumed. Fecal analyses showed that over 90 per cent of the excess food was absorbed. However, no data were submitted as to the efficiency of food utilization.—B. SURE

An Analysis of the Gain in Weight of Overfed Thin Young Men. B. Passmore, A. P. Meiklejohn, A. D. Dewar, and R. K. Thow. *Brit. J. Nutrition* 9: 27, 1955.

It is a common belief that thin people have some mysterious means of disposing of surplus food. While a proper understanding of these mechanisms is unlikely until many more metabolic experiments have been made on human subjects, it is felt that the observations presented in this communication may be found useful in planning future experiments. A study is presented of the balances of energy, nitrogen, water, sodium, and potassium, measured in these three men during overfeeding. Some inductions are made therefrom about the disposal in the body of the surplus food.

Three constitutionally thin young men were overfed under standard conditions for periods of 10 to 14 days. Their gain in weight was less than three-quarters of the gain that should have been expected had the excess calories in their diet gone to make "obese tissue" composed of fat, cellular material, and extracellular fluid. The gain in weight in these thin men appeared to be due entirely to deposition of fat and protein. There was no evidence of any gain in body water.—B. SURE

Origin and Treatment of Underweight in Girls. M. Albeaux-Fernet, E. Joaki, and J. Chabot. *Sem. Hôp. Paris* 34: 2151, 1954.

Under the name of "functional underweight," the authors describe some types of thinness frequently observed in girls, for which the point of departure is psychogenic but in which serious mental diseases and depressive states are absent.

The clinical and biologic diagnosis of these states is studied and their treatment is described. Hormones should not be employed; the essential factors on which the improvement of such cases of underweight depends are diet and psychotherapeutic treatment.—H. GOUNELLE

Losses of nitrogen, potassium, and vitamins in diarrheal states provide a nutritional study in humans with sprue or other gastrointestinal disturbances not found in experimental conditions.

Nutritional Deficiency Syndrome with Diarrhea Resulting in Hypopotassemia, Muscle Degeneration and Renal Insufficiency: Report of Case with Recovery. R. W. P. Achor and L. A. Smith. *Proc. Staff Meet., Mayo Clin.* 30: 207, 1955.

This report is a detailed history of a patient with a syndrome presenting features of pernicious anemia, sprue, and pellagra. Acute diarrhea resulted in a severe depletion of body potassium. A prolonged deficiency of this electrolyte resulted in marked renal insufficiency and acute degeneration of skeletal muscle.

With large amounts of potassium, protein, vitamins (including vitamin B_{12}), there was a good clinical recovery which has been maintained. According to the authors, a similar case had not been reported previously. —S. O. WAIFE

The following abstracts deal with various aspects of fasting and undernutrition in animals and humans. The possibility of increased adrenal activity in the fasted animal has been previously documented in the literature; this may account for the results described below.

Comparison of the Effects Produced by Fasting on Gross Bodily Activity of Wild and Domesticated Norway Rats. C. P. Richter and K. K. Rice. *Am. J. Physiol.* 179: 305, 1954.

During domestication, the adrenals become smaller and are observed to have less secretory activity. At the same time, rats are less resistant to stress. Furthermore, gonadectomy markedly decreases the activity of domesticated animals whereas wild rats are almost unaffected. Cortisone restores the decreased activity in gonadectomized domesticated rats. These experiments examine the effects of fasting on domesticated and wild Norway rats. An increased activity during fasting would increase the opportunity to find food.

Activity in the complete absence of food is more marked in wild than in domesticated rats, which would be to the disadvantage of the latter during famine. This may be explained in part by the increasing importance of the gonads over the adrenals during domestication. Domesticated rats become inactive after gonadectomy, but survive adrenalectomy with small amounts of salt, while wild rats die after adrenalectomy even though large amounts of salt are given. Higher amounts of adrenal cortical secretion in wild rats keep them active despite the gonadectomy. Fasting depresses the gonads but stimulates the adrenals. Therefore, in domesticated rats, fasting, which depresses the gonads, decreases activity. However, in the wild rat, it would not be effective. Fasting increases activity in the wild rat more than in the domestic because in the former the adrenals are larger and more active.—M. J. OPPENHEIMER

Stress of Fasting and Realimentation as Reflected in the Capillary Resistance and Eosinophile Count. J. Kramar, C. M. Wilhelmj, V. W. Meyers, D. P. Milani, D. E. Gunderson, D. Shuput, E. M. Racher, and P. S. Mahoney. *Am. J. Physiol.* 178: 486, 1954.

Fasting dogs were observed to have a progressive increase in capillary resistance and a decrease in circulating eosinophils. When realimentation with luxus diets high in carbohydrate was permitted, there was a tendency to maintain fasting levels of capillary

resistance and eosinophils. High protein diets, however, restored capillary resistance to control levels. Eosinophils returned more slowly, often with a marked initial overshoot. In the rat, capillary resistance increased during the fast. With the advent of realimentation, a very low level was attained. Eosinophils changed inversely with capillary resistance. Other forms of stress produce similar changes in the rat. In this form, realimentation with diets high in carbohydrate or protein had no effect upon capillary resistance.—M. J. OPPENHEIMER

Serum Lipoprotein Changes During Fasting in Man. L. Rubin and F. Aladjem. *Am. J. Physiol.* 178: 263, 1954.

After a 3–4 day fast in human volunteers, low density serum lipoprotein concentrations were significantly increased, but high density lipoproteins were unchanged. After 24 hours on a normal diet, normal levels were again observed. Sucrose administered at the end of the fast was without effect.—M. J. OPPENHEIMER

Serum Lipoprotein Changes During Fasting in Rabbits. F. Aladjem and L. Rubin. *Am. J. Physiol.* 178: 267, 1954.

Fasted rabbits had an increase in serum lipoproteins after three days. At the end of seven days, standard S_f 0–12 serum lipoproteins were increased the most. Standard S_f 12–20 and 20–100 lipoproteins were not increased over the three-day level. The standard S_f 100–400 lipoprotein group had decreased to the control level at the end of seven days.—M. J. OPPENHEIMER

SODIUM AND CARDIOVASCULAR DISEASE

The restriction of dietary sodium has been recognized as a fundamental feature of the therapeutic program in congestive heart failure. Many physicians have reported on benefits derived from the use of sodium restriction in patients with hypertension, although this has been a controversial point. The effectiveness of such diets in the treatment of edematous states or hypertension depends upon the ability of patients to adhere to the program, which, in turn, is a function of how well the physician is able to "sell" and teach it with or without the help of a dietitian. Variations in the actual sodium content of diets from those calculated from tables are discussed below.

The Sodium, Potassium and Chloride Content of the "200 mg. Sodium Diet." W. H. Hulet. *Am. J. Med. Sc.* 229: 85, 1955.

Thirteen separate menus prepared by a metabolic kitchen, each constructed to contain 200 mg of sodium and providing 70 g of protein, with calories ranging from 1900 to 2300, were analyzed for their electrolyte composition by a method described by the author. In addition, a sample menu of the Kempner rice and fruit diet was prepared for analysis. In the preparation of the diets, the tables of Bills *et al.* were employed. In the group of 13 diets analyzed, the actual sodium content was found to range from 304 to 812 mg, with an average of 513 mg. The Kempner diet was found to have 169 mg, rather than the 29 mg expected. It is suggested that the actual determination of the sodium content of diets is required, since there is some doubt as to the validity of the tables used for computing these values.—C. R. SHUMAN

Previous reports that salt withdrawal in hypertensives results in a lower salt excretion, urine volume, and weight loss than in control subjects are contradicted below. The reasons for the disparity are not apparent, so that further observations will be required.

Observations upon the Withdrawal of Sodium Chloride from the Diet in Hypertensive and Normotensive Individuals. R. Renwick, J. S. Robson, and C. P. Stewart. *J. Clin. Investigation* 34: 1037, 1955.

It has been stated that there is a clear difference in response of normotensive and hypertensive subjects to withdrawal of salt. The hypertensives lost less weight and excreted a smaller urine volume in the first 24 hours. In the present report on six hypertensive and six normotensive subjects on a constant diet, it was found that considerable individual variation in weight and urinary volume exists after abrupt salt withdrawal. However, no consistent difference between normotensive and hypertensive groups was found.

After salt withdrawal, and while urinary sodium excretion was diminishing, a temporary increase in urinary potassium excretion was found. There was no associated increase in nitrogen excretion and hence the potassium loss was not due to tissue breakdown. This suggests that increased adrenocortical activity may be an immediate response to the removal of salt from the diet.—S. O. WAIFE

The original Kempner diet appeared to have therapeutic effects ascribed to the sharp reduction of protein content as well as to salt restriction. Later work seemed to prove that sodium restriction alone was the important factor when protein was increased. Not all workers agree that these regimens are effective in lowering blood pressure; however, many feel that severe hypertensives should be treated with a combination of salt restriction and antihypertensive drugs. Differences in clinical experience are illustrated in the following two articles.

Effects of Diet in Essential Hypertension. III. Alterations in Sodium Chloride, Protein and Fat Intake. F. T. Hatch, A. R. Wertheim, G. H. Eurman, D. M. Watkin, H. F. Froeb, and H. A. Epstein. *Am. J. Med.* 17: 499, 1954.

This is the third article in a series of very extensive clinical experiments dealing with the effect of various

diets in the treatment of hypertension. In this particular publication, the effect of utilizing various techniques for the modification of the strict Kempner rice diet is described in 47 severe hypertensives.

As is always the case, the criteria for improvement were arbitrary, but consisted in this series of the following: (1) change in diastolic pressure of 10 mm Hg or more; (2) "definite" change in arteriolar spasm or pallor, retinal edema, hemorrhages or exudates, and papilledema; (3) change of 1 cm or more in transverse cardiac diameter; (4) definite changes in S-T segment and/or T waves; (5) change of 1 kg or more in weight; (6) Change of 10 mg per cent or more in blood urea nitrogen.

The experiments were divided into several groups. Thirteen patients were given, unbeknown to themselves, 3 g of salt in placebo capsules at the outset of the Kempner regime. This prevented the expected lowering of the blood pressure in all patients. However, with discontinuation of the added salt, four patients showed significant lowering of the pressure after six weeks. Seven patients showed no change, and two were worsened. There were equally equivocal results in the other findings noted.

The addition of 0.5 per cent g of salt to the diet of seven patients who had previously responded to the Kempner regime with significant decreases in pressure failed to produce any real change except in one patient. Salt was then added in stepwise fashion up to 3 g per day, and the authors feel that there was a correlation between increased salt and worsening of the findings.

The rice diet of nine patients who had responded favorably was then supplemented with low sodium protein substances up to 50 g of protein per day. There was no significant change in any of the findings, nor was there any after the addition of vegetables or vegetable oils.

The final observation was made on the effect of a specially prepared low sodium diet which contained approximately the same amount of sodium as the Kempner diet, but was much richer in protein. The results were as follows: (1) six patients who had responded to the rice diet showed no essential change when switched to the special diet; (2) one of three patients who had not responded to the rice diet did show a fall of 24 mm Hg in diastolic pressure while consuming the special diet; (3) of three subjects who were placed initially on the special diet, all showed significant improvement; (4) three of four patients who were followed at home on the special diet experienced a return of blood pressure to the previous level.

From this well-done work, it would appear that what has been suspected for a long time is true, namely, that the benefit of the rice diet lies in the fact that it is low in sodium content. However, the practical fact remains that it is a difficult task to persuade a patient to adhere to it faithfully, and if the same end is achieved by a more palatable diet, much will be gained. This seems to be true from this paper, but unfortunately the preparation of such a diet is, at best, time-consuming and expensive and cannot be generally available in every hospital. Another important fact, which is obvious from this publication, is that once improvement occurs, one can be a little more liberal with salt intake and still maintain the advantage.—J. F. MUELLER

Analysis of 177 Cases of Hypertensive Vascular Disease with Papilledema. B. Newboarg and W. Kempner. *Am. J. Med.* 19: 33, 1955.

During an eleven-year period 177 patients with malignant hypertension were evaluated for purposes of intensive dietary treatment with the "rice" diet. In six patients the observation period was too brief for adequate evaluation. In 33 patients with papilledema who were willing to follow the diet, electrolyte imbalance developed within the first three months, so that drastic salt restriction could not be enforced. In 31 of these patients, the total phenolsulphonphthalein excretion in two hours was less than 36 per cent. All 33 patients died. In 18 patients, treatment with the rice diet was not conducted; one of this group is still living. Treatment with the diet was conducted in 120 patients with hypertensive neuroretinopathy over a period of 1 to 117 months. In 100 patients eyeground photographs were available before and after variable intervals of treatment; in 92 of these patients, papilledema disappeared completely. There was partial to complete disappearance of hemorrhages and exudates in many instances. Hypotensive drugs were employed after imposition of the diet in 100 patients, resulting in further lowering of blood pressure but no change in the funduscopic picture. The effect of the diet upon blood pressure, serum cholesterol, cardio-thoracic ratio, and renal function was studied in 61 patients re-examined after at least one year of treatment. It is apparent from these examinations, as well as from the analysis of the fatal cases, that strictest adherence to the rice diet produces the most favorable results. Dietary modifications should be made gradually and with careful clinical observations. Intensive dietary therapy should be started early and continued until all signs of vascular disease have disappeared.—C. R. SHUMAN

The importance of sodium depletion as a harmful effect derived from prolonged salt restriction has become widely recognized recently. Decreased body sodium produced by excessive use of diuretics and salt restriction must be differentiated from dilution hyponatremia and intracellular sodium migration.

The Low Salt Syndromes. T. S. Danowski, E. B. Fergus, and F. M. Mateer. *Ann. Int. Med.* 43: 643, 1955.

This article is a short, concise review by an author who has contributed a great deal to the general problem of electrolyte balance. A suggestion is made that the term "low salt syndrome" may be misleading and

the term hyponatremia is offered in its place. A working classification of the hyponatremias is set forth as follows: A. hyponatremia with sodium stores decreased—this is the most common variety produced by either actual sodium loss or passage of the sodium ion into the cell or some body compartment, such as ascites or hypodermoclysis deposit; B. hyponatremia with sodium stores intact—water intoxication from various causes is the prime example of this variety, although it also occurs in patients with massive hyperlipemia; C. hyponatremia with sodium stores increased —this is the least well understood condition but is seen occasionally in seriously ill patients with cardiac, hepatic, or renal disease.

Means of differentiation are recorded from both the clinical and laboratory viewpoints. However, since these states are frequently mixed, one has to rely often on an old clinical trick—the therapeutic trial. Careful administration of sodium chloride with close observation of the weight and urinary output will frequently decide the issue.

The authors correlate hyponatremia with mortality and find in the adult it is a grave prognostic sign. In infants the mortality is not so high, but it is still considerable, so that every effort should be made to diagnose correctly and institute appropriate therapy as soon as possible.—J. F. MUELLER

The mode of action of corticotropin (ACTH) and cortisone in producing diuresis in edematous states has not been satisfactorily explained. A high level of the potent sodium-retaining hormone, aldosterone, has been found in these conditions. Is it possible that the electrolyte and water shifts induced by ACTH or steroid therapy act to suppress the elaboration of aldosterone by the adrenal cortex?

The Varied Patterns of Water and Sodium Diuresis during Corticotropin (ACTH) Therapy of the Nephrotic Syndrome. G. H. Heidorn, F. R. Schemm, and J. A. Layue. *Am. J. Med. Sc.* 229: 180, 1955.

Seven patients with the nephrotic syndrome were treated with ACTH and balance studies for sodium and potassium were performed. Estimated weight changes were calculated from sodium balances and compared to actual weight in order to estimate the proportionate water to salt diuresis or retention for a given period. Varied patterns of water and sodium balances were observed in these patients. The loss of edema fluid was greatest usually beginning 24 hours after ACTH was stopped. In most instances, sodium was lost in excess of water. However, proportionate loss of water and sodium or an excess of water over sodium loss were also observed in the post-ACTH period. Weight gain, when observed during the course of treatment, was due more to water retention than to sodium retention. The responsiveness of nephrotic or cardiac patients to mercurial diuretics can be regained after or during a course of ACTH administration. The qualitative response of the tubules to mercurial diuretics is altered by ACTH in that a water diuresis occurred early in the treatment period, while a salt diuresis in excess of water occurred later in the course of hormonal therapy. —C. R. SHUMAN

Body Sodium and Potassium. IV: The Normal Total Exchangeable Sodium; Its Measurement and Magnitude. I. S. Edelman, A. H. James, L. Brooks, and F. D. Moore. *Metabolism* 3: 530, 1954.

The intravenous injection of 100 microcuries of radiosodium (Na^{24}) provides a convenient and accurate means of estimating the exchangeable sodium content of the body using the dilution formula: Total exchangeable sodium = (Na^{24} injected–Na^{24} excreted)/(serum Na^{24}/ serum Na^{23}). Sodium exchange in man has been studied with reference to skeletal and smooth muscles, gastric mucosa, and erythrocytes; in animals, these tissues and various organs were examined. As judged by the serum and tissue specific activity ratios, equilibrium of distribution was complete 15 to 24 hours after injection. In human subjects, observations on serum specific activities revealed that a constant value is reached 12 to 18 hours after the intravenous administration of Na^{24}. There is apparently no advantage in extending the period of equilibration beyond 24 hours. The mean total exchangeable sodium for males was 40.9 meq per kg and for females 37.8 meq per kg of body weight. The combined data from the literature for both sexes and over a wide age range is relatively constant and is approximately 41.8 meq per kg.—C. R. SHUMAN

Interesting observations have been reported on the elevation of sodium within the walls of arteries obtained from hypertensives. In sodium-depleted animals, the lowering of blood pressure is correlated with low sodium content of these vessels.

Effect of a Low Sodium Diet on Electrolyte Composition of Arterial Wall. L. Tobian, Jr. *Am. J. Physiol.* 181: 599, 1955.

Normal rats were maintained on a low sodium diet. After a regime like this the sodium content of the aorta is reduced 12 per cent but the phosphorus content is significantly elevated. In these animals there is a very small increase in serum sodium. The water, magnesium and potassium content of the aorta was unchanged when compared to those of a control group. Sodium restriction reduced the blood pressure insignificantly. The author is of the opinion, however, that the low sodium content of the aorta after restriction of sodium in the diet may be related to the tendency for blood pressure to be lower in such rats.—M. J. OPPENHEIMER

Desoxycorticosterone is capable of producing hypertension and of increasing tissue sodium content. A high level of sodium feeding alone may produce these changes in

experimental animals. It is apparent that the salt-retaining hormone accelerates this effect under experimental conditions, although, as the other papers show, the relationship is not very simple.

Increased Sensitivity to Salt in Rats Pretreated with Desoxycorticosterone. F. M. Sturtevant and N. Hassen. *Am. J. Med. Sc.* 229: 188, 1955.

Rats receiving desoxycorticosterone and saline developed hypertension within a few days. Upon withdrawal of both the hormone and saline drinking fluid, normotension was restored promptly. However, readministration of the isotonic saline resulted in the reappearance of hypertension in the DCA-treated animals. It is suggested that experimental hypertension induced by DCA is an accelerated form of the disease produced by high salt intake alone. The elevation of blood pressure is a consequence of a more fundamental disturbance in the regulation of electrolyte distribution and excretion.—C. R. SHUMAN

Role of Adrenal and Hypophysis in Regulation of Sodium Excretion. D. H. Simmons, R. B. Harvey, and T. Hoshiko. *Am. J. Physiol.* 181: 379, 1955.

In these experiments it was observed that large doses of desoxycorticosterone acetate do not produce results such as are brought about by restricted sodium intake. Moreover, hypophysectomy caused only very small changes in renal sodium-retaining activity. These changes are by no means of the same degree as those which result from marked alterations in sodium intake in the conditions of this study. The absence of the pituitary had only a minor influence on the responses observed in the dog when sodium intake was varied widely. Balance studies were used to make this aforementioned evaluation. It is suggested that neither the adrenal cortex nor the pituitary gland are needed for the regulation of sodium excretion. The possibility that both these endocrine glands may have a non-regulatory function is, however, not excluded.—M. J. OPPENHEIMER

Mechanisms of Desoxycorticosterone Action. X. Effects on Tissue Sodium Concentration. D. M. Green, T. B. Reynolds, and R. J. Girerd. *Am. J. Physiol.* 181: 105, 1955.

For the most part, DCA increases tissue sodium. Skeletal muscle show the greatest increase if the route of administration is pellet implantation. Nephrectomy blocks this action. The level of sodium exchange plays the most important role in determining the action of DCA in relation to tissue sodium. It was observed that an amount of DCA which was effective during high sodium exchange was ineffective when this exchange was low. The relation of DCA action in elevating tissue sodium to that under which it is a hypertensive agent was emphasized.—M. J. OPPENHEIMER

RICKETS, VITAMIN D, AND CALCIUM

The current recommendation of the Food and Nutrition Board of the National Research Council for minimum daily vitamin D requirements is 400 IU for infants, children, and adolescents. In premature infants, pregnant and lactating women, and special conditions in children, the requirement is raised to 3000 IU daily. In adults, there is no indication that supplemental vitamin D is required. The action of vitamin D may be that of increasing phosphorylative activity within the cells of the intestinal mucosa, renal tubules, and bone. In vitamin D deficiency resulting in rickets, an increased rate of urinary amino acid excretion has been described. The administration of vitamin D decreased the aminoaciduria according to several reports.

Some Observations on Rickets. J. H. P. Jonxis. *Glasgow M. J.* 36: 227, 1955.

In this interesting and well-written lecture, the author reviews the history of rickets and explores some unsolved problems in this disorder. The disease was first described in the 17th century in England and apparently spread over Europe gradually, descending the social ladder. How a change in housing, religion (Lent), and clothing may have acted to spread rickets is described.

At the University of Groningen the author's group found that some babies excreted an increased amount of free amino acids in the urine. They also showed evidence of rickets. Administration of vitamin D returned the aminoaciduria to normal. In a few severe cases the urine levels did not completely return to normal values and these children were members of families with a high incidence of rickets. The author believes that an increased need for vitamin D and an increased aminoaciduria are both hereditary, associated and concomitant.

There is some evidence that amino acid excretion in the urine with moderate rickets is on one end of a scale, with the de Toni-Fanconi syndrome on the other, and "resistant rickets" in the middle. Vitamin D reduces the huge excretion of amino acids in the de Toni-Fanconi syndrome.

Certain renal studies indicate that the increased tubular reabsorption of histidine and arginine, following their intravenous administration, interferes with the reabsorption of similar amino acids, such as threonine, serine, glycine, and lysine. No evidence that the parathyroids are involved could be found to support Albright's theory of secondary hyperparathyroidism in rickets. It is clear, however, that vitamin D is necessary for the normal action of some enzyme system needed for the reabsorption by the kidney of phosphates and amino acids at least.—S. O. WAIFE

Methionine-Induced Amino-Aciduria in Vitamin D Resistant Rickets. W. H. Fishman. *Metabolism* 4: 107, 1955.

Two children with vitamin D-resistant rickets were given methionine orally for a period of ten days, before and after which the urinary amino acids were determined. It was found that an increased excretion of amino acid and of hydroxyamino acids occurred after methionine administration. It is suggested that serine accounts for the excess of hydroxyamino acids.—C. R. SHUMAN

The calcium requirements for children have been published from many sources. During the pubertal growth spurt, the retention of 300 to 400 mg daily of calcium requires an intake of approximately 1.4 g per day. Vitamin D is necessary for the utilization of calcium in promoting the absorption of 20 to 25 per cent of dietary calcium during this period. Additional aspects of vitamin D and calcium metabolism are discussed in the following papers.

Vitamin D and Calcium Requirements. H. E. Harrison. *J. Am. Dietet. A.* 31: 483, 1955.

The author reviews our knowledge concerning vitamin D and calcium requirements of humans and comments on the possibility of deleterious effects of excessive calcium intakes. It is pointed out that the best assumption concerning vitamin D need is that it is required throughout life and that requirements of the older child and adult are no greater than those of young infants. Available evidence seems to indicate 400 IU vitamin D daily will satisfy needs of all age groups. Osteomalacia may occur in adults with disturbances of fat absorption and increased loss of fat in the stools, since vitamin D is not absorbed and calcium is unavailable because it is bound to fatty acids as insoluble soaps.

Vitamin-D enriched milk supplies 400 IU vitamin D per quart. When milk consumption falls below this amount, additional sources must be sought. Food sources are egg yolk, tuna fish, salmon, and herring. Breast-fed infants require supplemental vitamin D, as human milk is not rich in this vitamin.

Studies of calcium requirements based on maximal calcium retentions give rise to some questions; e.g., a breast-fed infant consuming a quart of human milk makes good gains and receives 300 mg calcium daily; an infant receiving cow's milk, which contains 4 times the amount of calcium in human milk, grows faster and retains more calcium than the breast-fed infant. Are bones of the cow's milk-fed infant stronger than those of the breast-fed? The author states that it is more likely that bones can vary within fairly wide limits with regard to density of calcium salts and still be normal in structure and growth.

The growth rate of American children during the past few decades has been greater than in previous generations. Though high calcium intakes from infancy on may play a role in this growth acceleration, other dietary improvements, such as protein content, vitamin B-complex, and ascorbic acid intake cannot be ignored as possible causative agents.

In answer to the question as to whether excessive calcium intakes can be harmful, the author refers to studies in which it was reported that *adults* with peptic ulcer taking high amounts of milk and alkalinizing agents showed hypercalcemia and kidney injury; *infants* in England and Scotland were reported to have excessive levels of serum calcium on cow's milk feeding. It is further pointed out that in this country high calcium diets and vitamin D supplements are fed to large numbers of infants without evidence of hypercalcemia or symptoms due to excessive urinary calcium. In summary, the author indicates that a majority of American children receive a diet adequate with respect to calcium and vitamin D. Occurrence of rickets, so common 2–3 decades ago, is now a rarity. Efforts to promote adequate intake of milk and milk products by children should not be slackened, though we can be satisfied that great progress has been made in the more adequate provision of calcium in diets of American children.—J. M. SMITH.

Disturbances in Calcium and Phosphorus Metabolism. G. Fanconi. *Metabolism* 4: 95, 1955.

Impairment of renal function will result in several disturbances in phosphate and calcium metabolism. The first lesion described is that of glomerular insufficiency leading to hyperphosphatemia and hypocalcemia. This alteration produces secondary hyperparathyroidism with bone resorption, known as renal osteodystrophy. The second condition described is one in which hypercalcemia and hyperphosphatemia are found in association with azotemia, osteosclerosis, and dwarfism. The cause of this condition is not clear; the basic disturbance may reside in the kidney or in the central nervous system. The condition known as chronic hyperparathyroidism is discussed in terms of a primary disturbance in the midbrain or in the target organs, rather than to changes in the function of the parathyroid glands. The term "chronic constitutional hypocalcemia" has been suggested for hypoparathyroidism and associated disorders. The lesion originally described by the author (the Fanconi syndrome) is discussed as a renal tubular defect associated with an abnormally high phosphate excretion by the kidneys. In this condition, there is chronic aminoaciduria and glycosuria with hypophosphatemia. Renal tubular acidosis is discussed as a defect in the regulatory mechanisms in the excretion of electrolytes.—C. R. SHUMAN

The calcium requirement in individuals in older age groups has recently become the subject of several studies. Interest in this aspect of geriatric nutrition stems partly from the frequency of osteoporosis and fractures occurring in these groups. It has been shown that older individuals tend to lose calcium and that increased calcium feeding may reduce this loss. Whether calcium retention affects ossification favorably or whether extra-osseous calcification is promoted is not known. With respect to the bones,

it is likely that the loss of calcium occurring during the process of aging is due to changes in the protein-rich bone matrix.

Calcium and Phosphorus Utilization in Older Men. K. Daum, W. W. Tuttle, A. Weber, M. T. Schumacher, and J. Salzano. *J. Am. Dietet. A.* 31: 149, 1955.

Seven men varying in age from 53 to 83 years served as subjects for this study, details of which were given earlier in a report on the effects of omission of breakfasts on physiologic response (*J. Am. Dietet. A.* 28: 117, 1952). Over a period of nine months the men ate weighed diets during periods designated as "basic bacon, egg, and milk breakfast," "no breakfast," and "basic cereal and milk breakfast." These breakfasts provided one-fourth of the total daily caloric and protein allowance. During the "no breakfast" period, food was omitted between 6 p.m. and noon on the following day, and the daily food allowance was eaten in two meals instead of three. Individual diet patterns were determined by free choice of the subjects prior to the control period. Four weeks were allowed for adjustment before data were collected, with four weeks between experimental periods. Collection of urinary and stool specimens were made only during the fourth week of each experimental period. Thus, this study reports data on calcium and phosphorus intake and excretion of seven subjects during one week of the three experimental periods. Individual and mean data are recorded.

Calcium intakes ranged from 1.463 to 2.621 g daily, and phosphorus from 1.607 to 2.771 g. The quantity for each subject was quite constant during the three periods.

Positive calcium retention was found in all subjects during the cereal-and-milk and no-breakfast periods. Two subjects showed negative calcium balances in the third period. The mean daily calcium retentions of 0.062, 0.238, and 0.230 for the bacon-egg-milk, no-breakfast, and cereal-and-milk breakfast periods, respectively, were slightly less than previously observed by the authors in a similar experiment with young men. These differences were not statistically significant, however.

Positive phosphorus balances were recorded for all but two of the 21 individual values. The most consistent retentions were in the no-breakfast periods. The authors state that on the basis of group means, the daily intakes and balances for calcium and phosphorus were not significantly different in the breakfast regimes studied.

It is of interest to the reviewer that these older men voluntarily chose such generous amounts of calcium. In view of the paucity of recorded calcium balance data for older men, it would have been of further interest to see several more periods for each subject in order to note whether there was any trend toward greater or smaller retentions of calcium during the prolonged periods of generous calcium intake.—J. M. SMITH

It has been suggested in the past that the thyroid hormone affects the renal threshold for calcium. This may explain the increased calcium retention noted at lower basal metabolic rates. Interesting data on calcium utilization are also noted.

Further Experiments on the Utilization of Calcium from Salts by College Women. M. B. Patton. *J. Nutrition* 55: 519, 1955.

In an earlier study on the utilization of calcium from four salts, there were indications that the order in which the salts were taken was a factor in the utilization of calcium from these sources. The present study was planned to give a subject the same salt for a period of six weeks with the objective of determining whether or not she would utilize the calcium from the salt equally well from week to week. The earlier study also gave an indication of a relationship between calcium utilization and basal metabolic rate. The present study was designed to give further consideration to this relationship.

The calcium balances of nine college women consuming approximately 600 mg of calcium were analyzed. Approximately one-half of the calcium consumed came from foods commonly served in American diets; the other half was supplied by milk in three subjects, calcium carbonate salts in three subjects, and calcium gluconate salts in the remaining three. Statistical analysis of the data showed that under the experimental conditions, milk, calcium gluconate, and calcium carbonate were utilized equally well by these subjects as a source of calcium. Differences in balance from week to week were significant, but the relative importance of the time factor was reduced during the final two weeks, the 5th and 6th periods, from that in the preceding periods.

The basal metabolic rate was inversely related to calcium utilization. In general, the basal metabolic rates of the subjects were lower at the close of the study than at the beginning and this may be related to the greater utilization during the final two weeks.—B. SURE

The condition known as idiopathic hypocalcemia has recently been ascribed to an increased susceptibility to the action of vitamin D. There is also the possibility that some of the patients with this disorder have received a maintenance dose of vitamin D which is in excess of their needs. The toxic effects of hypervitaminosis D are readily reversible in these individuals if the vitamin is stopped in time.

Calcium Metabolism in Idiopathic Hypercalcaemia of Infancy with Failure to Thrive. R. E. Bonham Carter, C. E. Dent, D. I. Fowler, and C. M. Harper. *Arch Dis. Child.* 30: 399, 1955.

It has been suggested that the syndrome which has become known in Britain as idiopathic hypercalcemia with failure to thrive, may be due either to excessive

vitamin D or else to a hypersensitivity to vitamin D.

A boy of nine months suffering from the milder form of the disease which began some 2 or 3 months earlier, was intensively investigated in a metabolic ward. When the disease was in remission, 10,000 units of calciferol by mouth daily caused an acute exacerbation. On a vitamin D- and calcium-free diet, plasma calcium level returned to normal and there was marked clinical improvement. On a diet containing 610 mg Ca daily, other things remaining constant, plasma Ca rose to abnormal levels and the child became ill again. The clinical and metabolic disorders closely mimicked a vitamin D intoxication, in this case presumably due to an abnormal sensitivity to the vitamin.

The child eventually made a spontaneous recovery and was able to tolerate a normal diet. His growth was normal and he was well two years later.

On the basis of these experiments it is suggested that this syndrome be treated with a low calcium, vitamin D-free diet, or else a normal diet with cortisone to antagonize the vitamin D.—F. E. HYTTEN

Intake of Vitamin D in Infancy. R. D. G. Creery, and D. W. Neill. *Lancet* 2: 372, 1955.

Since the recognition of the syndrome of "idiopathic hypercalcemia" in British infants and its possible relationship to vitamin D, it has been suggested that many infants are getting more vitamin D than they need, and this survey is the first attempt to examine the situation.

Information about feeding and vitamin supplementation was collected on 1087 three-month-old Belfast infants and represented about 90 per cent of all three-month infants in the city at the time.

Only 19.5 per cent of the infants were being either wholly or partly breast fed, 16.2 per cent were having liquid cow's milk, 32 per cent proprietary brands of dried milk, and 32.3 per cent National Dried Milk; the vitamin D intake is shown for each group.

Overall, 10.8 per cent had no added vitamin D, 15 per cent had less than 600 IU daily, 19 per cent 600—1000 IU daily, and 51 per cent between 1000 and 2000 IU daily; 4.2 per cent received more than 2000 IU daily.

Those having less than 600 IU were almost all breast fed or receiving liquid cow's milk and those with the higher intakes were mostly having dried milk feeds—the highest having vitamin supplementation in addition. Methods of achieving a better balance in the community are briefly discussed. It is suggested that breast-fed infants and those fed on liquid cow's milk should always have supplementary vitamin D, and that the vitamin D content of dried milks and cereals should be reduced, supplementary vitamin also being reduced in infants fed on dried milks.—F. E. HYTTEN

The hexaphosphoric acid ester of inositol, phytic acid, is found in cereal grains; it combines with dietary calcium to form insoluble calcium phytate. The following papers indicate mechanisms of vitamin D action in increasing the availability of calcium and phosphorus.

The Role of Vitamin D and Intestinal Phytase in the Prevention of Rickets in Rats on Cereal Diets. V. J. Pileggi, H. F. De Luca, and H. Steenbock. *Arch. Biochem. & Biophys.* 58: 194, 1955.

It has been generally agreed that vitamin D increases the availability of phytate phosphorus. The authors have previously reported that there was a definite trend toward an increase in intestinal phytase when rats and chicks were given vitamin D. In the present study, the effect of vitamin D on the *in vivo* hydrolysis of dietary phytate and on the *in vivo* activity of phytase and phosphatase in fecal and intestinal extracts was studied.

It was noted: (1) that rats on basal low calcium cereal diets hydrolyzed almost all ingested phytate regardless of presence or absence of vitamin D. (Hydrolysis was estimated by difference between ingested and fecal phytate phosphorus.) Addition of $CaCO_3$ markedly lowered the hydrolysis. Further addition of vitamin D raised it, but not to level attained in absence of $CaCO_3$. When vitamin D was not present the hydrolysis of phytic acid was reduced from 99 per cent to 51 per cent by addition of 1 per cent $CaCO_3$, and to 27 per cent by addition of 3 per cent $CaCO_3$. When vitamin D was present the corresponding values were 67 and 43 per cent; (2) that vitamin D increased the activity (both of phytase and phosphatase) of extracts from both intestines and feces. This increase always occurred whether animals were on cereal or non-cereal, rachitogenic or non-rachitogenic diets; (3) that no consistent correlation in a quantitative sense could be found between degree of hydrolysis of dietary phytate and enzyme activity nor with severity of rickets. In addition, (4), by considering the available phosphorus (P) to be the sum of dietary non-phytate P and the fraction of phytate P which did not appear in feces, they calculated the available P to be 0.145 per cent of the ration when vitamin D was not given and 0.185 per cent when the vitamin was fed. When these amounts of P were fed in the form of inorganic salts as the sole source of P, rickets was produced unless vitamin D was added. Finally (5) more inorganic P was lost in feces of rats not receiving vitamin D, even though these animals hydrolyzed less phytate.

From these results the authors consider that the antirachitic action of vitamin D with cereal diets is due primarily to an improved utilization of inorganic phosphorus.—M. K. HORWITT

Calcium Absorption and Vitamin D. B. B. Migicovsky and J. W. S. Jamieson. *Canad. J. Biochem. & Physiol.* 33: 202, 1955.

The mode of action of vitamin D in promoting calcium absorption has not been satisfactorily established.

Using chicks, the authors found that with oral Ca^{45},

absorption was increased by vitamin D, whereas calcium administered intramuscularly appeared in bone irrespective of vitamin D. Vitamin D increased the proportion of oral calcium absorbed by a constant factor, so that the amount absorbed depended to a considerable extent on the amount of calcium ingested. The effect of vitamin D was greater following a period of low calcium intake than after a period of high calcium intake.

The authors conclude that the action of vitamin D may be to form a complex with calcium, ferrying it across the gut and facilitating its deposition in bone.—F. E. HYTTEN

In general, calciferol in large doses is capable of maintaining normal serum calcium levels in parathyroidectomized patients if administered with calcium salts. Rarely, it has been necessary to administer dihydrotachysterol (A.T. 10) because of the failure of calciferol to produce normocalcemia. One patient originally unresponsive to calciferol responded well to A.T. 10 but later was found to be controlled with doses of vitamin D_2 which were initially ineffective.

Insensitivity to Vitamin D Developing During the Treatment of Postoperative Tetany. Its Specificity as Regards the Form of Vitamin D Taken. C. E. Dent, C. H. Harper, M. E. Morgans, G. R. Philpot, and W. R. Trotter. *Lancet* 2: 687, 1955.

Four cases are described in which, after accidental surgical removal of their parathyroid glands during operations on the thyroid gland, the patient had chronic hypocalcemic tetany. All showed a greatly increased insensitivity to the hypercalcemic action of calciferol, even though they had been previously normocalcemic on smaller doses.

When the same or smaller doses of dihydrotachysterol (A.T. 10) (3 cases) or pure vitamin D (1 case) were substituted for the calciferol, the plasma calcium returned to normal and the patients became symptomless. All four have been well maintained for long periods on small doses of these substitutes.

This curious phenomenon is likely to have some bearing on the pathogenesis and treatment of those various forms of bone disease which include so-called "resistance to vitamin D" as one of their features.—F. E. HYTTEN

The Effect of A.T. 10 on Calcium and Phosphorus Metabolism in Resistant Rickets. P. D. Saville, J. R. Nessim, F. H. Stevenson, L. Mulligen, and M. Carey. *Clin. Sc.* 14: 489, 1955.

There has been considerable confusion in recent literature about the relative merits of calciferol and dihydrotachysterol (A.T. 10) as anti-rachitic substances.

Metabolic balance studies are presented of six patients with resistant rickets treated with A.T. 10. In all cases there was a rapid decrease in the fecal excretion of calcium and phosphorus. In five cases (aged 5 to 15 years) there was not a corresponding rise in urinary excretion, so that a positive calcium and phosphorus balance was achieved. In the sixth case, a woman of 41 and mother of two of the other cases, the urinary excretion rose in proportion to the increased absorption and she remained in balance.

When sodium phosphate was added to the diet, all balances of calcium and phosphorus became more strongly positive.

The authors conclude that A.T. 10 is qualitatively and quantitatively similar to calciferol and that differences which have been described can probably be attributed to differing metabolic states at the time of test. They also postulate that the poor absorption of calcium often seen in resistant rickets may be due primarily to a low serum inorganic phosphorus which is insufficient for calcium to be precipitated into bone.—F. E. HYTTEN

The possibility of removing calcium from extra-osseous sites of calcification by the use of a chelating agent which will increase the renal excretion of the ectopic salt requires further study. Perhaps the binding of calcium within the mucopolysaccharide structures of the kidney and other areas is less stable than within bone. Adequate laboratory tests are sorely needed in this field.

The "in vivo" Dissolution of Metastatic Calcium. An Approach to Atherosclerosis. N. E. Clarke, C. W. Clarke, and R. E. Mosher. *Am. J. Med. Sc.* 229: 142, 1955.

The disodium salt of ethylene diaminetetraacetic acid (EDTA) was administered intravenously as a solution of 5 g of the salt in 500 cc of 5% glucose or saline. Patients with nephrocalcinosis, coronary artery disease, peripheral vascular disease, calcific mitral stenosis, and other diseases associated with calcium deposition in tissues were treated in this fashion. The average number of 50 injections were given per patient. The toxic manifestations of this treatment included a burning sensation at the site of infusion, nausea and diarrhea and abdominal cramps in several patients; dermatitis and mucous membrane lesions developed in nine patients. The administration of pyridoxine was noted to reduce the incidence and severity of the toxic reactions. Normal serum calcium levels were maintained during the treatment period. Roentgenograms are shown which demonstrate removal of significant amounts of calcium from the renal parenchyma of a patient with nephrocalcinosis. Otosclerosis was favorably affected, with a striking improvement in hearing being noted after EDTA treatment. No statement is made concerning its effect upon angina pectoris or the other diseases treated.—C. R. SHUMAN

Failure of Calcium Tolerance Test as a Diagnostic Aid in Malignant Osteolytic Disease. L. E. Meiselas and M. G. Goldner. *Metabolism* 4: 250, 1955.

Previous studies have indicated that the calcium excretion rates following a calcium load are increased above normal in osteolytic lesions and are decreased in the presence of osteoblastic lesions. In the present work calcium excretion rates were determined before, during, and after an intravenous calcium load in 14 patients with and without cancer. Cancer patients were found to excrete significantly greater calcium loads than control patients after intravenous calcium administration. However, excessive calcuria was not found to be diagnostic for osseous lesions. The excretion patterns were not altered by the presence of multiple bony metastases, so that it was considered unlikely that obscure lesions would be detected by the test. Furthermore, there was a wide variability in the results of repeated testing of the same individual.—C. R. SHUMAN

The Use of Radioactive Phosphorus in the Assay of Vitamin D. P. Numerof, H. L. Sassaman, A. Rodgers, and A. E. Schaefer. *J. Nutrition* 55: 13, 1955.

The validity of the radiophosphorus procedure for vitamin D assay reported by previous investigators has been confirmed. The method has been compared against the line test in concurrent assays and has given potencies agreeing with those obtained with the U.S.P. standard line test. The radiophosphorus assay has been shown to possess several advantages over the U.S.P. "line test." The prime advantage is that the subjective element in the line test assay is completely eliminated, without any loss in accuracy. Furthermore, complications inherent in the line test, such as calcification evidenced as a diffuse accretion contiguous to the diaphysis, decreased food consumption, and insufficient weight gain, do not appear to affect the results when the radiophosphorus method is used.—B. SURE

THIAMINE METABOLISM

It is known that thiamine acts primarily in pyruvate utilization as the pyrophosphate ester, cocarboxylase. In this relationship, thiamine requirements are increased during the metabolism of carbohydrate. Despite this fact, dietary thiamine requirements are conventionally considered in terms of total calories rather than carbohydrate intake. If fat is substituted for carbohydrate in the diet, a thiamine-sparing effect has been observed.

The Influence of Dietary Fat on Thiamine Loss from the Body. L. E. Holt, Jr. and S. E. Snyderman. *J. Nutrition* 56: 495, 1955.

Studies carried out in human infants revealed that the substitution of carbohydrate for fat in the diet resulted in a diminished output of thiamine in the urine. This is interpreted as indicating an increased demand for thiamine in metabolic reactions. The authors, thus see no need to question the accepted explanation for the thiamine-sparing action of fat of Gershoff and Hegsted (*J. Nutrition* 54: 609, 1954).—B. SURE

Studies continue to appear concerned with the effect of cocarboxylase injection. It has been questioned in these columns whether or not phosphorylated compounds of this type are rapidly dephosphorylated in the blood stream by phosphatase. Investigations of this nature should be carefully controlled with thiamine and inert substances.

Influence of Cocarboxylase on the Urinary Excretion of 17-Ketosteroids, 11-Oxycorticoids, and 11-Desoxycorticoids. Investigations in Normal Subjects. P. Larizza, L. Suardi, and F. Gobbi. *Acta vitaminol.* 8: 214, 1954.

Intravenous cocarboxylase (phosphorylated thiamine) has been shown to produce a number of hematologic and metabolic effects resembling those observed after ACTH or cortisone: eosinopenia, modifications of the lymphocyte and neutrophil levels (decreased and increased, respectively), and increased uric acid/creatinine ratio. Suggestive, too, of a possible affinity between these three substances is their analogous action in bronchial asthma. Against this background, the authors report the effect of cocarboxylase on excretion of urinary metabolites of adrenal cortical origin.

After a single intravenous injection of 50 mg of cocarboxylase, 24-hour urinary 17-ketosteroids increased (average +18.66%) in 12 of 20 cases, while in the others they decreased (average −13%). The clinical response of asthmatics to cocarboxylase follows a similar pattern: in some subjects a response occurs like that to ACTH or cortisone, in others there is no reaction or an opposite effect. Urinary 11-desoxycorticoids increased (average +30%) in 13 of the 20 cases, diminished (average −18%) in the rest. The response of the 11-oxycorticoids was more uniform: an increase (average +47%) occurred in 75 per cent of the 20 cases, a decrease (average −18%) in the remaining 25 per cent.

After 5 days of treatment with 50 mg/day of cocarboxylase, 17-ketosteroid excretion of 8 normal subjects showed similar variations, except that in all but one of the 8 there was an increase after the second and third dose, after which the response became more irregular, as if adrenal-cortical reactivity to cocarboxylase (assumed) has been exhausted. With regard to 11-oxycorticoids and 11-desoxycorticoids, the pattern of response was again similar, with the most striking elevation occurring in the case of the 11-oxycorticoids. This would suggest that any effect cocarboxylase may have on the adrenal cortex is exerted primarily on the zona fasciculata. However, the authors believe that the available evidence is still too meager to permit any conclusion as to (a) whether the observed effect of cocarboxylase is specific or common to other phosphorus donors (such as ATP); or (b) whether its action on the adrenal cortex is direct or indirect (mediated by the pituitary). Certainly the hypothesis cannot be advanced that cocarboxylase *per se* has cortisone-like properties independent of direct or indirect cortical participation.—C.-J. HOWELL

Thiamine deficiency resulting in beriberi is improved by thiamine administration; however, correction of the clinical status requires the feeding of a balanced dietary intake. Similar results may be observed in reactions involving specific enzymes in certain experimental animals.

Dietary Conditions Affecting Oxidation of Tyrosine by Rat Liver, with Particular Reference to Thiamine. J. P. Kring and J. N. Williams, Jr. *Proc. Soc. Exper. Biol. & Med.* 87: 97, 1954.

Oxidation of tyrosine by the tyrosine oxidase system from livers of thiamine-deficient animals is higher than in normal animals. The same condition can be brought about by inanition. The condition is not corrected by thiamine feeding unless a complete ration is fed as well.—L. W. KINSELL

The prolonged Q–T interval and the changes in the T–wave observed in the electrocardiogram in vitamin B_1 deficiency have been attributed to alterations in potassium metabolism. Low tissue potassium has been described in experimental animals which have been made deficient in thiamine. The electrocardiographic changes described below in patients with beriberi are similar to those observed in potassium deficiency.

The Electrocardiogram in Oriental Beriberi. R. A. Pallister. *Trans. R. Soc. Trop. Med. & Hyg.* 48: 490, 1954.

Most of the reports on the changes in the electrocardiogram in beriberi have come from North America, the observations having been made on chronic alcoholics. Records from the East are relatively rare. This paper describes studies made in 25 patients with beriberi in Penang, Federation of Malaya. The diagnosis was based on the association of peripheral neuritis with heart failure, and rapid response to vitamin B_1. Seven of the cases were considered to be acute; the others were mild. In sixteen patients the ECG was abnormal: the commonest change was inversion of the T-wave, usually most marked in the chest leads taken over the right ventricle. Prolongation of the Q–T interval was also common. Other occasional findings were depression of the S–T interval and premature beats. All these changes sometimes became more marked, or appeared for the first time, after treatment had been begun.

These abnormalities in the ECG, while not specific, tend to form a pattern which may be of some help in diagnosis. They are more common in older patients and are related, as might be expected, to cardiac enlargement. They are essentially similar to those reported in so-called occidental beriberi in Europe and North America. The reason why some authors have given the impression that changes in the electrocardiogram are uncommon in oriental beriberi may be that the patients in the East tend to be younger.—J. WATERLOW

FLUORINE—ESSENTIAL TRACE ELEMENT?

Concentrations of fluorine in drinking water of approximately 1 part per million have been shown to lower the incidence of dental caries in many of the population groups tested. This observation, in addition to the finding of small amounts of fluoride in the skeletal tissues, is the basis for considering fluorine as an important trace element in nutrition. This element acts during the calcifying period of the teeth to produce a hard enamel with reduced solubility in the presence of the cariogenic acids.

Fluorine in Food and Drinking Water. Dental Health Benefits and Physiologic Effects. F. J. McClure. *J. Am. Dietet. A.* 29: 560, 1953.

An extensive series of epidemiologic studies and supporting laboratory and experimental data during the past 25 years have established the beneficial effect of fluorine drinking waters on dental health.

Studies of caries incidence in communities where communal waters naturally contained fluorine have revealed dental caries reduction by as much as 60 per cent in population groups aged 12 to 14 years who have been exposed to natural fluoride waters throughout life. Roughly 4.5 per cent of the population in the United States has access to natural fluoride water containing 0.5 p.p.m. or more of fluorine.

Studies of artificially fluoridated drinking water, started in 1945, are beginning to show similar benefits. Grand Rapids, Michigan, maintained at a level of 1.00 p.p.m. since January 1945 (sodium fluoride as a carrier) showed in the fall of 1951 a reduction in dental caries rate in permanent teeth from 66.6 per cent in 6-year-old children to 18.1 per cent in the 16-year age group. Similar results were obtained for deciduous teeth. At the present writing controlled fluoridation provides approximately 14 million people with drinking water averaging 1.00 p.p.m. of fluorine. Among the large cities with a fluoridation program are Washington, D. C., Baltimore, Indianapolis, and San Francisco.

The author presents an extensive list of fluorine contents of common foods. He estimates that the average daily fluorine intake may be approximately 1.2–1.5 mg from drinking water (1.00 p.p.m.) and 0.2–0.30 mg from food.

The general belief that fluorine (1.00 p.p.m.) can be incorporated in public water supplies with no unfavorable effects on the public is based on clinical and epidemiologic observations on human subjects exposed to fluorine and, to a lesser extent, on controlled studies on a variety of animals. Fluorine characteristically accumulates in skeletal and dental tissues; thus prolonged periods of excessive fluorine ingestion may be unsafe unless ingested fluorine is adequately eliminated from the body. Extensive studies with animals and humans have shown that fluorine in trace quantities is largely eliminated by normally efficient urinary excretion.

Studies of the effect of fluorine on the health status of individuals living in areas of the United States where natural fluoride waters have been used have shown no failure or impairment except dental fluorosis. Similar height-weight data and bone fracture experiences have been found for boys and young men living in fluoride and non-fluoride areas. Upwards of 4.0 to 4.5 p.p.m. fluorine in drinking water had no effect on calcification of carpal bones of children 6 to 13 years of age. Among a group of 114 adults (average age for men 43 years and for women 46 years) exposure to as much as 8.0 p.p.m. fluorine in their drinking water for 20 years showed no systemic complaints related to the fluorine water and evidences of incipient skeletal change as revealed by x-ray examination in only 7 of them.

The author emphasizes that though there is no doubt that fluoridation of drinking water at a level of 1.00 p.p.m. is an adequate public health practice for partial control of dental caries, it is not justifiable to anticipate total abolition of dental caries through its use. Dental research has produced no adequate answers as to causes of dental caries—further knowledge of causative factors may provide additional measures for more complete caries control. At best only 65 per cent of the population in the United States uses a communal water supply.

In the light of the evidence available, and in the absence of characteristic lesions in teeth and bones, it may be safely assumed that the hazard of chronic fluorosis does not exist. Nonetheless, there is need for continued research designed to elucidate further the public health aspects of fluoridated drinking water.—J. M. Smith

Toxicity of fluorine is evidenced as chronic dental fluorosis or mottled enamel, and probably in certain areas as otosclerosis. Acute poisoning occurs usually in relation to its use in industry and in insecticides.

Waterborne Fluorides and Mortality. T. L. Hagan, M. Pasternack, and G. C. Scholz. *Pub. Health Rep.* 69: 450, 1954.

This paper compares mortality rates from all causes and from five selected causes—heart disease, cancer, intracranial lesions, nephritis, and cirrhosis of the liver—in 64 cities, 32 having fluoride in their water in concentrations of 0.70 p.p.m. or more, and 32 cities having less than 0.25 p.p.m. The cities were paired, each fluoride city with the nearest non-fluoride city. No statistically significant difference could be found between the mortality rates of the two groups of cities.—M. W. Bates

Fluorine is widely distributed in nature; in bonemeal added to baby foods a high concentration of the element is found. Information concerning fluorine metabolism and its nutritional significance is meager. The balance studies reported below are important additions to the literature.

Fluorine Balance Studies on Four Infants. M. P. Ham and M. D. Smith. *J. Nutrition* 53: 215, 1954.

The present balance studies were undertaken in order to obtain data on the amount of fluorine ingested by infants and its absorption and retention, if any. Four normal, healthy male infants served as experimental subjects and a fifth was studied in a preliminary experiment. Two balance periods, each of three days' duration, were completed on each infant. In the first period, the infants were 6 to 14 weeks old and received a milk formula only. In the second period, they were 16 to 18 weeks old and received a milk formula plus 9 to 12 tablespoons of the baby food, Pablum, per day. The method used was, with some modification, essentially that outlined for the determination of fluorine in foods in the Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists.

With the exception of fluorine which may occur in drinking water, bonemeal-containing cereals, such as Pablum, seem to be the chief source of fluorine in the infant diet. Pablum is often fed to the infant at the age of 3 months or even sooner. The level of fluorine in the daily diet of a 4-month old infant taking a milk formula plus Pablum was found in these studies to range from 476 to 706 micrograms, an amount as high or higher than that found by previous authors in the much larger amount of food normally consumed by adults.

The addition of Pablum to the infants' diets considerably increased the amount of fluorine ingested. Generally, the studies indicated that the fluorine in infants' diets was retained to some extent. The per cent retention appeared in each case greater in period II than in period I, and the absolute amounts retained were also higher.—B. Sure

Fluorine Balance Studies on Three Women. M. P. Ham and M. D. Smith. *J. Nutrition* 53: 225, 1954.

To learn more about the absorption of fluorine and its retention, the authors carried out balance experiments of three young women. Tea infusion to contain approximately 1 p.p.m. fluorine was added to the diet in one of the periods as a source of fluorine occurring in a soluble form. In another period, the diet was supplemented by Pablum (a bonemeal-containing baby food) which contributed fluorine associated with calcium and in a relatively insoluble form. The young women were studied during three periods which were three days each in length. In period I, the subjects consumed a normal diet; in period II, a diet which included approximately 70 g of Pablum per day; and in period III a diet which included 1360 to 1815 ml of tea per day. The levels of fluorine ingested during periods II and III were relatively the same and considerably higher than on the normal diet in period I.

There was retention of fluorine by all three subjects in each of these three periods. The fluorine ingested in period II was absorbed to a lesser extent than that ingested in period III. It thus appears that the fluorine

in Pablum is not so available for absorption as that in tea.—B. SURE

Metabolism of Fluorine 18 in Domestic Animals. J. D. Perkinson, Jr., I. B. Whitney, R. A. Monroe, W. E. Lotz, and C. L. Comar. *Am. J. Physiol.* 182: 383, 1955.

In the lamb the initial rate of disappearance of isotopic fluorine (F^{18}) which had been injected intravenously was 41 per cent of the dose per minute. The comparable figure in the mature cow was 32 per cent. Absorption is very rapid and is probably from the rumen. One per cent of the ingested dose represented the maximum value observed in the blood. The peak came earlier in the lamb at about two hours after the material was ingested, while in the cow the peak was at five hours. Bone and mandible autoradiograms showed that radiofluorine was selectively localized in a manner resembling that of calcium. Some ingested F^{18} was observed to have been deposited in hen's egg and cow's milk.—M. J. OPPENHEIMER

IODINE DEFICIENCY

Evidence has been provided to show that a low concentration of iodine in ingested foods and waters within certain geographical areas will result in hyperplasia of the thyroid gland. This response on the part of the gland is presumed to be the result of an increased stimulation to thyroidal cell growth and activity arising from the anterior pituitary. This gland releases more thyrotropic principle due to the lack of suppression by circulating thyroxine.

Goitre in Ceylon and Nigeria. D. C. Wilson. *Brit. J. Nutrition* 8: 90, 1954.

This paper describes the incidence of endemic goiter in the island of Ceylon and in the territory of Nigeria and relates the incidence to diet, to quality of drinking water, and to geological formation. In the wet areas of Ceylon, where the incidence of enlarged thyroids in schoolgirls is high, the iodine content of drinking water is low (1.4 to 2.7 μg/liter) and the usual diet poor. In Nigeria, the distribution of goiter is associated with water supplies of very low iodine content (0.6 to 0.7 μg/liter) from pre-Cambrian granites. In both areas a lack of iodine in food and water appeared to be of primary etiological importance.—B. SURE

Endemic Goiter in Latin America. N. S. Scrimshaw. *Pub. Health Rep.* 69: 282, 1954.

In 1950, endemic goiter was recognized as a serious health problem in Latin America. Treatment with potassium iodate and iodized salt were successful in reducing the incidence of goiter. Since potassium iodide is unstable in hot humid climates, potassium iodate was used also. The drop in goiter incidence was similar when either substance was used. One part of iodine to 10,000 parts of salt was suggested as a standard to be used in Latin America which can be furnished either by potassium iodide or iodate.—M. W. BATES

Goitre and the Iodine Content of Cow's Milk. W. T. Binnerts. *Nature* 174: 973, 1954.

There are marked regional and seasonal differences in the iodine content of cow's milk. The following figures were found in a recent Netherlands investigation:

Area	Mean iodine content of milk (μg/100 ml)	
	Summer	Winter
Goitrous	0.97 (0.026)	2.06 (0.053)
Transitional	1.38 (0.047)	3.21 (0.13)
Non-goitrous	2.11 (0.077)	4.88 (0.15)
Extreme coastal	3.43 (0.21)	8.35 (0.59)

The figures in parentheses represent the standard errors of the means.

The author suggests that milk analysis is the most useful method for surveying the "iodine status" of cattle.—F. E. HYTTEN

The increased P^{32} uptake noted in the thyroid glands of iodine-deficient animals suggests that cellular activity is enhanced by the pituitary agent, thyrotropin.

Uptake of P^{32} by Chick Thyroid and Testes Following Pituitary Administration, Iodine Deficiency and Thiouracil. W. L. Money and R. W. Rawson. *Am. J. Physiol.* 182: 1, 1955.

A single injection of a pituitary preparation was administered to chicks. This substance contained thyroid-stimulating hormone and gonadotropins. When this injection was followed by P^{32} an increased concentration of the isotope was detected in thyroid and testes. A group of chicks were maintained on a restricted iodine intake or were given thiouracil. In these experimental subjects there was an observed increase in thyroid P^{32}. However, gonadal uptake of the isotope was increased in animals receiving a low iodine diet but not in those to whom thiouracil was administered. If the pituitary preparation was administered to animals on a low iodine diet, P^{32} uptake by thyroid was increased over that of a control group on ordinary diet.—M. J. OPPENHEIMER

Nutritional Availability of Iodine from Several Insoluble Iodine Compounds. S. Mittler and G. H. Benham. *J. Nutrition* 53: 53, 1954.

The availability of iodine from insoluble iodine compounds is important in the selection of an iodine source to be incorporated into an animal salt block. An insoluble iodine source is desirable, in that iodine would not be leached out readily by exposure to moisture. The availability of iodine from cuprous iodide, from diiodo-

dithymol and from 3-5-diiodosalicylic acid was compared to the availability of iodine from potassium iodide.

Iodine was found to be nutritionally available from insoluble cuprous iodide, diiodothymol, and 3-5-diiodosalicylic acid. Acute and chronic toxicity tests indicated that cuprous iodide and 3-5-diiodosalicylic acid were non-toxic in amounts required to protect the thyroid gland.—B. SURE

We must be aware of various routes of iodine elimination; such factors are recalled by the following report.

The Excretion of Radioactive Iodine in Human Milk. H. Miller and R. S. Weetch. *Lancet* 2: 1013, 1955.

A case is reported of a 23-year-old woman who was given 29.5 μc of I^{131} as a test of thyroid function. The woman was lactating and her milk was withdrawn for 48 hours and its radioactivity measured. About 1.3 μc appeared in the milk during this time, and the concentration in the milk was considerably higher than in the mother's plasma.

It is considered that the risk to the infant of this dose is not negligible and that radioactive iodine should not be given to nursing mothers.—F. E. HYTTEN

ITEMS OF GENERAL INTEREST

Extraolfactory Components of Flavor. E. C. Clark and H. W. Dodge, Jr. *J. A. M. A.* 159: 1721, 1955.

This interesting study was based on five patients whose olfactory nerves had been surgically proved to be destroyed, either by tumor or trauma. Compared to 10 normal controls, all subjects had an intact sense of taste.

Blindfold tests were made with 31 vegetables and fruits so prepared as to prevent easy identification. Although no anosmic patient did as well as the best normal controls in identifying test foods, some of the patients without a sense of smell did as well as the controls, and in fact, better than the poorest control.

From the observations presented in detail, it is apparent that extraolfactory components at times play a major role in flavor identification. These components include somatic sensation in the mouth and nasopharynx, as well as vision, hearing, memory, and the psychologic state at the time. The possibility exists that some undiscovered receptive mechanism in the mouth and nasopharynx may also be a factor in flavor perception. The authors suggest that for this concept of flavor the term "savor" is more appropriate.—S. O. WAIFE

Clinical and Chemical Studies in Human Lactation. X. The Maintenance of Breast Feeding. F. E. Hytten and A. M. Thomson. *Brit. M. J.* 2: 232, 1955.

The breast feeding performance was studied of all married women in the city of Aberdeen delivered of single children in 1951. Seventy-six per cent were delivered in the Aberdeen Maternity Hospital, 7 per cent in private nursing homes, and 17 per cent in their own homes. The incidence of full breast feeding was 35.6 per cent at the end of three months and 24.5 per cent at the end of six months after delivery, figures which are similar to those reported from elsewhere in Britain. The figures were analyzed in terms of maternal age, parity, physique, and social class. There was a positive correlation between age and the incidence of breast feeding at three months in all parity and social class groups: this contrasts markedly with the negative correlation with age of the incidence of breast feeding on discharge from hospital—the crossover occurs at about one month.

Breast feeding performance was better with second babies than with first babies, but thereafter declined.

Women from the upper socio-economic levels maintained breast feeding better than those from lower groups, and tall women and women in good general health maintained breast feeding more successfully than shorter women and women of poorer physique.

In general, once breast feeding is established, its maintenance is largely determined by good maternal health and favorable social circumstances. These are closely interrelated and imply a wide range of advantages, from good physique and diet to good education and sensible attitudes. The relative contributions of all these factors to the promotion of breast feeding are difficult to determine and lines for future research are suggested.—F. E. HYTTEN

On the Presence of a Deficiency Factor in the Pathogenesis of Amyotrophic Lateral Sclerosis. E. Ask-Upmark and S. Meurling. *Acta Med. Scandinav.* 152: 217, 1955.

According to the authors, there may be a deficiency factor in the pathogenesis of amyotrophic lateral sclerosis. This is based on the finding of a "higher" incidence of the disease in the absence of the stomach. Thus, of 111 cases of gastric resection for peptic ulcer, one case (and possibly two more) of the disease was found. Out of 20 cases of amyotrophic lateral sclerosis seen over a 10-year period, five had had a gastric resection, two had diabetes and emaciation, and one had pyloric stenosis. The authors believe that malnutrition of some type may be involved. It would seem, however, that this is skating on the thin ice of *post hoc, ergo propter hoc* reasoning.—S. O. WAIFE

The Severity of Aortic Arteriosclerosis in Certain Diseases: A Necropsy Study. D. L. Creed, W. F. Baird, and E. R. Fisher. *Am. J. Med. Sc.* 230: 385, 1955.

An evaluation of the severity of aortic arteriosclerosis observed in 1223 necropsies performed on males at a Veterans Administration Hospital over a 7-year period is reported, in order to determine the influence of a

variety of diseases upon the process. The sclerotic process was found to be more severe in patients succumbing to hypertension and bronchogenic carcinoma than in control patients. The aortas of patients with malignant lymphoma or leukemia revealed a statistically significant decreased severity of arteriosclerosis in the range of 51 to 60 years. A similar decrease in sclerosis was noted in patients with Laennec's cirrhosis. The mechanism of increased arteriosclerosis in patients with bronchogenic carcinoma is unexplained. The decreased severity in cirrhosis may be related to the clearing action of estrogen upon serum lipoprotein; failure of estrogen inactivation in chronic liver disease is well known. In addition, the presence of jaundice is associated with an increase in serum phospholipids which aid in stabilization of serum lipid emulsions. The hepatic involvement noted in patients with malignant lymphoma and leukemia may account for the decreased severity of arteriosclerosis on the same basis as in cirrhosis. The prolonged poor nutritional status which many of these patients exhibit is associated with a reduced severity of the sclerotic prcess.—C. R. SHUMAN

Problem of Softened Water in Low Salt Diets. W. A. Sodeman and D. Meyer. *Missouri Med.* 52: 783, 1955.

In many areas of the United States hard water (high calcium and magnesium content) is the rule. It is common practice to use water softeners in the homes. Many of these are resins which exchange their sodium for the calcium and magnesium in the water. A study was made of the sodium content in water obtained from university buildings in Columbia, Missouri. Samples from a single source showed great variability in sodium values, depending on care and maintenace of the water-softener units. In a number of instances, the sodium content was high enough to interfere with maintenance of a rigid low sodium intake. A liter commonly contained 160 to 170 mg of Na. Obviously, the amount of water used in drinking and cooking will determine the ultimate sodium intake. The authors recommend (1) use of unsoftened water for cooking and drinking, and (2) use of distilled water if even the untreated hard water contains significant amounts of sodium.— S. O. WAIFE

Effect of Restriction of Dietary Fat and Cholesterol upon Serum Lipids and Lipoproteins in Patients with Hypertension. F. T. Hatch, L. L. Abell, and F. E. Kendall. *Am. J. Med.* 19: 48, 1955.

Restriction of dietary fat and cholesterol was imposed upon a series of hospitalized patients with essential hypertension. The restriction of dietary fat to 3 g per day and elimination of cholesterol intake did not reduce serum lipids and lipoprotein levels below the normal range of values. The body is capable of maintaining the mechanisms operating in the production of serum lipids and lipoprotein when dietary fat is not available. Carbohydrate and protein sources operate to support the endogenous synthesis of lipids present in the serum. The results of drastic dietary restriction of fat and cholesterol showed great variability among patients in the effects on serum lipid patterns. With fat intake below 40 g, the serum cholesterol esters declined; free cholesterol and lipid phosphorus showed no change. However, 20 per cent of these patients exhibited lipemia due to the elevation of serum neutral fat. Dietary restriction did not alter the concentration of the S_f 20–100 class. The response in any given patient to fat restriction was not predictable. There was no correlation between the observed changes and the severity of hypertension and arteriosclerotic complications. Restriction of fat rather than cholesterol is responsible for the variations seen in cholesterol and lipids in the serum. Hypersensitivity to dicumarol was noted during fat restriction, attributable to faulty absorption of vitamin K.—C. R. SHUMAN

The Relationship of Cirrhosis of the Liver to Hypertension: A Study of 504 Cases of Cirrhosis of the Liver. H. F. Loyke. *Am. J. Med. Sc.* 230: 627, 1955.

The nature and frequency of hypertension in a total of 567 cases of cirrhosis of the liver was studied. The diagnosis of cirrhotic disease was based on the clinical diagnosis, biopsy specimens, and necropsy findings; blood pressures were evaluated with the patient in recumbency. The average blood pressure referred to age groups shows that there is an absence of the progressive increase with age which has been described in normal individuals. Hypertension was found to be unusual in cirrhotic patients. The renal and neurogenic mechanisms responsible for elevating the blood pressure in normal subjects remain intact in cirrhosis of the liver. Stressful stimuli were found to produce transient hypertension in cirrhotics. Renal disease occurring in cirrhotics was capable of producing a sustained hypertension. Previous hypertension was shown to be reversed to normal pressure with the establishment of cirrhosis. During the reversal of hypertension, coincidental reversal of the A/G ratio was noted. The question of an abnormal globulin in maintaining or blocking "essential" hypertension is discussed.—C. R. SHUMAN

Body Lipids in Small Mammals Following Prolonged Exposures to High and Low Temperatures. D. R. Young and S. F. Cook. *Am. J. Physiol.* 181: 72, 1955.

Male Swiss mice were maintained at various environmental temperatures for 30 to 36 days. Temperatures utilized in the experiments were 4°, 24°, and 35° C. At the end of this time the animals were sacrificed and analyzed for total lipids. Those maintained at 24° C were considered as controls. At the lower temperature, mice consumed more food than controls. However, these animals did not grow and less total lipids were deposited than in the case of the control group. On the other hand, at high environmental temperatures, less food was used but growth and fat deposits resembled

control values. After similar experimental conditions for periods up to 150 days, Long-Evans rats were examined for skin and carcass fatty acids. At low temperatures, food intake increased but growth and fat deposits were less. At high temperatures, less food was consumed but growth and fat deposits were normal. Melting points of carcass fatty acids went up slightly as environmental temperatures mounted. Skin fatty acid melting points were unchanged, no matter what environmental temperature obtained.—M. J. OPPENHEIMER

Precipitation by Fatty Food of Systemic Disturbance from Argentaffinoma. N. M. Bleehen. *Lancet* 2: 1362, 1955.

Some patients with argentaffin carcinoma have paroxysmal attacks of flushing, dyspnea, and diarrhea. The onset of symptoms has been related to the secretion by the tumor of 5-hydroxytryptamine (5-HT) and a variety of external factors have been suggested as precipitating these attacks.

A case is described of a 64-year-old woman who was subject to severe attacks of this nature from 5 to 15 minutes after eating a "fatty cooked meal" for 4 years before the diagnosis of the tumor. The association between the intake of fatty food and the secretion of 5-HT was demonstrated during investigation.—F. E. HYTTEN

The Metabolism of Methylated Aminoazo Dyes. IV. Dietary Factors Enhancing Demethylation in Vitro. R. R. Brown, J. A. Miller, and E. C. Miller. *J. Biol. Chem.* 209: 211, 1954.

At least one N-methyl group is required for the carcinogenic activity of 4-dimethylaminoazobenzene and related dyes in the liver of the rat. The N-methyl groups are readily metabolized. With 3-methyl-4-monomethylaminoazobenzene as the substrate in a rat liver homogenate system, one can account quantitatively for the substrate disappearing as 3-methyl-4-aminoazobenzene and formaldehyde. Further studies of this system showed that the ability of rat liver homogenates to N-demethylate 3-methyl-monomethylaminoazobenzene depended on the nature of the diet fed. Thus the livers of mice fed a commercial dog chow had twice the demethylation activity of those from mice fed grains or purified diets. Similar activities were found in rat livers.

The factor responsible for the increase in activity was also noted in a number of aged or otherwise treated animal products, such as old cholesterol preparations, liver extracts, and peptones. A variety of pure sterols were inactive, but could be made active by peroxidation. A number of other peroxides, including fatty acid peroxides, were inactive. The evidence presented indicates that the factors present in the various crude tissue derivatives are not present in fresh tissues, but are generated, probably from sterols, during processing and storage.—M. K. HORWITT

Life Span of "Yellow" Mice Fed Enriched Diets. R. Silberberg, M. Silberberg, and S. Riley. *Am. J. Physiol.* 181: 128, 1955.

In the strain of mice under observation, females lived longer than males. High fat diets shortened the life span. The effect was more marked in yellow coated mice than in gray, and more marked in males than in females. Overweight produced by carbohydrate did not change the life span.—M. J. OPPENHEIMER

Removal and Mobilization of Lipids in Normal and Hepatectomized Dogs. J. J. Spitzer. *Am. J. Physiol.* 181: 83, 1955.

In these experiments, "functional hepatectomies" were performed by ligation of the portal vein, hepatic artery, and common bile duct. Protamine increased the lipemia in normal animals. The increase was less marked and less consistent in normal or functionally hepatectomized dogs in whom the thoracic duct was cannulated. There is a marked increase in visible lipemia after the portal vein and hepatic artery are tied. This effect is more pronounced in the femoral artery than in the femoral vein. Protamine increases this lipemia and heparin clears it. After protamine the increases in the lipemia are more marked in the femoral artery than in the vein. Visible changes in lipemia are accompanied by chemical changes in blood lipid. These experiments are considered to support the premise that changes produced by heparin and protamine depend on removal and mobilization of lipids.—M. J. OPPENHEIMER

Effects of Cortisone and Epinephrine Exhibition on Lipid Components and Phospholipid Turnover in Plasma, Liver and Aorta of Rabbits. A. Dury and N. R. Di Luzio. *Am. J. Physiol.* 182: 45, 1955.

Rats were given single injections of epinephrine daily for 14 days. Another series received cortisone and a third a combination of cortisone and epinephrine. A control series was also studied. Plasma and liver lipids were increased by the exhibition of cortisone with or without epinephrine. These same combinations increased plasma and liver phospholipid synthesis. Aortic lipid concentrations and aortic phospholipid turnover were unchanged by the use of cortisone. The changes in plasma lipids after epinephrine were only moderate. Increased incorporation of isotopic phosphorus in the aorta was observed after epinephrine.—M. J. OPPENHEIMER

Incorporation of Acetate into Fatty Acid by Liver Slices of Adrenalectomized Rats. W. F. Perry and H. F. Bowen. *Am. J. Physiol.* 180: 21, 1955.

These experiments deal with the incorporation of 2-C^{14} acetate into fatty acids. In this study liver slices from adrenalectomized rats incorporated less acetate than did normal rats. This was true whether the

adrenalectomized rats were fasted, force-fed, or given an *ad libitum* diet. The subnormal formation of fatty acids from acetate (in the absence of the adrenals) can be ascribed to the relative unavailability of metabolites due to limited gluconeogenesis, and in some cases also to an insufficient food intake.—M. J. OPPENHEIMER

Influence of Fatty Acids and of the Adrenal Cortex on the Course of Oxygen Consumption with Changing Temperature. J. M. O'Connor. *Am. J. Physiol.* 181: 89, 1955.

This study deals with the influence of temperature on oxygen use in rat skin. Oxygen consumption increases in peaks from 15° to 38° C, with many variations. The peaks are seven in number and are observed to be related to seven naturally occurring saturated fatty acids. These range from caproic to stearic. Adrenalectomy lowers the peaks but the amount varies with the different acids. Three peaks of the lowest acids are no longer demonstrable, but are restored by cortisone. Cortisone, deoxycorticosterone and laparotomy tend to restore the peaks. Administration of corticotropin or epinephrine or cold stress have similar effects in intact but not in adrenalectomized animals. This article discusses the physical state (i.e., monolayer) of the fatty acids in the body.—M. J. OPPENHEIMER

Effect of Restriction of Dietary Sodium on Electrolyte Composition ot the Contents of the Terminal Ileum. H. Field, Jr., R. E. Dailey, R. S. Boyd, and L. Swell. *Am. J. Physiol.* 179: 477, 1954.

On a diet of low sodium bread, the fecal sodium concentration in the terminal ileum was markedly decreased. This observation is interpreted to mean that the amount of sodium presented to the stomach and intestine is severely restricted; the upper gastrointestinal tract conserves this ion. However, under these experimental conditions, fecal potassium in the terminal ileum was increased. This tends to maintain a constant osmolarity of ileal contents. Involved in this process one must consider selective absorption or secretion, or possibly a combination of the two factors. Evidence was also presented that the colon does not conserve potassium with any efficiency, since it does not absorb increased amounts which are presented to it in ileal contents.—M. J. OPPENHEIMER

Effect of Salt Depletion and Fasting on Water Exchange in the Rabbit. Kee-Chang Huang. *Am. J. Physiol.* 181: 609, 1955.

In this study, albino rabbits were subjected to a regimen of salt depletion and also of reduced food intake. Under these experimental conditions observations were made on water intake and urine volume. Extracellular electrolytes were depleted by intraperitoneal dialysis. As a result of this treatment all the animals showed a marked depression. A reduction in water intake was observed for the first day which followed the dialysis. Plasma chlorides and plasma volumes were reduced but plasma proteins were increased. Polydipsia and polyuria were later present in all but one animal during the first or second bout of salt depletion. There was a gradual increase in plasma sodium and chloride during simple fasting. Half the animals fasted were observed to have an increased water intake and urinary output. These last conditions were similar to those observed in the animals in whom salt depletion was carried out. It should be pointed out that in dogs fasting usually results in decreased fluid turnover.—M. J. OPPENHEIMER

Water and Electrolyte Content of Tissues of the Adrenalectomized and Adrenalectomized-Nephrectomized Dog. A. Grollman. *Am. J. Physiol.* 179: 36, 1954.

Adrenalectomized and adrenalectomized-nephrectomized dogs were maintained for from 20 to 40 days by intermittent peritoneal lavage. No hormones were used for maintenance. Sodium in variable amounts entered intracellular space, while potassium tended to leave the same space to a smaller degree. Changes in skeletal muscle and in the intestine were accentuated by nephrectomy.—M. J. OPPENHEIMER

Sodium and Potassium Exchanges Associated with Nor-Epinephrine Infusions. E. E. Muirhead, A. Goth, and F. Jones. *Am. J. Physiol.* 179: 1, 1954.

Nor-epinephrine infusions were carried out in dogs for periods of from 20 to 50 minutes. The dose was 1–7 mg/kg. This produced a marked hypertension. The plasma sodium level was depressed and that of potassium elevated. The chloride changes were not significant.—M. J. OPPENHEIMER

Relationship of Sodium Retention to Potassium Excretion by the Kidney During Administration of Desoxycorticosterone Acetate to Dogs. D. S. Howell and J. O. Davis. *Am. J. Physiol.* 179: 359, 1954.

Desoxycorticosterone acetate (DCA) was administered to normal dogs. When the sodium intake was moderate or high, a negative potassium balance was observed and the plasma potassium fell. However, when the sodium intake was low, these changes were not seen. Other animals were prepared with constriction of the thoracic inferior vena cava and placed on a moderate sodium intake. In this case potassium balance and plasma potassium were unchanged after DCA. It is pointed out that almost complete reabsorption of sodium and chloride in the renal tubules proximal to the site of sodium and potassium exchange might explain the failure of urinary potassium to increase following DCA administration.—M. J. OPPENHEIMER

Distribution of Injected Potassium Salts in Tissues of the Potassium-Deficient Rat. R. Schwartz, J. Cohen, and W. M. Wallace. *Am. J. Physiol.* 182: 39, 1955.

Young albino rats (Hisaw) were fed diets containing less than 1 mM of potassium per kg. They were then treated with potassium salts for 12 hours, after which the potassium-deficient regimen was restored. When potassium was fed there was an immediate gain in weight expressed mainly as growth. Water and electrolyte were retained early and there was a more delayed increase in body fat after potassium was administered. Muscle, which had a 35 per cent reduction in K during the period of dietary restriction, was restored to normal K concentrations within 5 hours after K was returned to the diet. At the same time, muscle sodium was observed to decrease about as much as cellular K increased. If the K salt was acetate instead of Cl, a hypertonicity resulted and some animals did not survive. There were no deaths if KCl was the salt used. —M. J. OPPENHEIMER

Effects of Potassium and Cortisone on Morbidity and Hyponatramia Following Mitral Valve Surgery. R. A. Bruce and K. A. Merendino. *Am. J. Med. Sc.* 230: 392, 1955.

Patients undergoing mitral valvulotomy have occasionally manifested an unexpected morbidity during the first postoperative week. Evidence of an increased total body sodium and water with hyponatremia, renal conservation of sodium and water, and a decreased exchangeable potassium has been presented. The effects of potassium and cortisone upon the morbidity and metabolic responses of 12 patients subjected to such operative procedure are described in this report. The administration of potassium was associated with the appearance of mild hyperkalemia in three patients given the chloride salt. Of the nine patients given cortisone, one exhibited mental symptoms; none developed either hyponatremia or hyperkalemia when fluid and sodium intake were limited. Cortisone was regarded as a useful adjunct in the treatment of circulatory collapse with pressor amines in one patient. It was concluded that fluids and sodium administration should be limited in the postoperative management of patients undergoing mitral valve surgery. Potassium should be given only as fruit juices or beef broth, as tolerated by the patient, rather than as supplemental potassium salts. Cortisone may be of value in occasional patients experiencing profound stress. It did not prevent pericarditis in five of the nine patients treated with this agent.—C. R. SHUMAN

Electrolyte Shifts and Electrocardiographic Changes During Tourniquet Shock in Rats. H. A. Ravin, J. R. Denson, and H. Jensen. *Am. J. Physiol.* 178: 419, 1954.

Tourniquet shock was produced in a standard manner in rats. After lethal shock and removal of the constrictions, the injured muscles were observed to have a rapid accumulation of extracellular fluid and intracellular sodium. Intracellular potassium fell progressively but calcium and magnesium were not significantly changed. The heart muscle lost sodium and gained potassium, although calcium, magnesium, and water were unaltered. When voluntary muscle was uninjured, the changes after less severe shock were similar to those stated above. When edema was forming rapidly in damaged tissues, the serum sodium was briefly depressed. Serum potassium was greatly elevated in the terminal phase. Serum calcium was elevated early after the tourniquets were released, but the magnesium level was variable. Late in shock, the electrocardiogram presented changes similar to those seen with elevated potassium. Injection of potassium was poorly tolerated, although the myocardium was still sensitive to this ion. The authors stress the depletion of intracellular sodium as a mechanism in the genesis of shock.—M. J. OPPENHEIMER

A Physiologic and Clinical Study of Failures in Vitamin A Metabolism in Tuberculous Patients. H. R. Getz. *Am. Rev. Tuberc. & Pul. Dis.* 72: 218, 1955.

A relationship between nutrition and tuberculosis has been known in a general way for many years. In this study 78 patients with moderately advanced pulmonary tuberculosis and low blood levels of vitamins A and C were placed on bed rest, an ample diet, and specific vitamin supplementation. None received chemotherapy, surgical, or collapse therapy for their disease. Signs and symptoms of vitamin A deficiency were found in patients with normal carotene and vitamin A ester concentrations, which suggests that these patients failed to convert carotene to vitamin A, and to release the vitamin from its ester form.

The control group, 37 subjects, subsisted on the same diet as the experimental group and received placebos in like manner. Another group, 26 patients, received large doses of synthetic vitamin A; the third group, 15 subjects, were given vitamin A in a crude cod-liver oil concentrate.

The diet, which contained a caloric excess (3000), an abundance of protein (120 g), and more than average amounts of vitamins A and D, did not raise the vitamin plasma levels significantly. A slow and irregular response in vitamin A levels was noted in the supplemented groups. This was most clearly seen in the subjects taking the cod-liver oil concentrate. Clinical failure (stationary or advancing disease, failure of cavity closure, development of complications) occurred in 15 of 37 controls, in 10 of 26 synthetic vitamin A-supplemented subjects, but in none of 15 crude concentrate-supplemented subjects. These results were statistically very significant.

From this long-term study the author concludes that a factor favorable to healing is present in the crude concentrate of vitamin A from cod-liver oil—S. O. WAIFE

Effects of a Combined Deficiency of Vitamins E and B_6 on Blood Picture of Rat. J. S. Dinning, J. M. Young, Jr., M. R. Simmons, and P. L. Day. *Proc. Soc. Exper. Biol. & Med.* 85: 280, 1954.

Rats maintained on diets deficient in both vitamins E and B_6 exhibited marked granulocytosis. This condition is prevented by supplementing the diet with either vitamin E or B_6. Creatine excretion followed essentially the same pattern as did the granulocyte level in the peripheral blood. None of the animals developed anemia.—L. W. KINSELL

Intestinal Length in Man. B. M. L. Underhill. *Brit. M. J.* 2: 1243, 1955.

The purpose of this report is to show that there is a wide variation in the length of the intestine in man and that it is not enough, when a long length of the gut is resected at operation, for the remainder to be inferred from reference to some average length.

The intestinal lengths of 65 men and 35 women were measured at autopsy. The length of the large gut was relatively constant at about $5^1/_2$ feet, but the small intestine varied in the extremes from 11 feet to about 26 feet. The length appeared to be correlated with height.—F. E. HYTTEN

Alteration of Rabbit Marrow Fat in Anemia from Acetylphenylhydrazine. J. D. Evans, J. M. Baker, and M. J. Oppenheimer. *Am. J. Physiol.* 181: 504, 1955.

From the alteration of marrow fat after poisoning with acetylphenylhydrazine, it is concluded that an active transport system exists for the preferential removal of linoleic and linolenic acids from marrow. Even though the eventual fate of the polyunsaturated fatty acids is still obscure, they may play an important role in body metabolism.—M. J. OPPENHEIMER

Comparative Studies on the Occurrence of the Carnosine-Anserine Fraction in Skeletal Muscle and Heart. G. Schmidt and R. Cubiles. *Arch. Biochem. & Biophys.* 58: 227, 1955.

Because of the divergence of figures reported on concentrations of carnosine and anserine in heart muscle, and especially because of the unexpectedly high values recently reported by Parshin and Gorynkhina (values as high as 100 to 150 mg per 100 g fresh heart tissue, based on quantity of primary amino groups liberated by action of a kidney peptidase on protein-free filtrates of heart tissue), an investigation of the problem, utilizing the techniques of paper electrophoresis and paper chromatograpny, was carried out.

In paper electrophoresis studies, both carnosine and anserine travel rapidly toward the cathode. Both bases give brown spots with ninhydrin easily distinguished from amino acid spots. Measurable amounts of the carnosine-anserine fraction (separation of the two bases was not achieved) was not found in heart muscle filtrates. It is possible that minute amounts of these bases may be present in heart muscle, but the results are incompatible with the high values reported by Parshin *et al.*—M. K. HORWITT

The Content of Riboflavin and Riboflavin-containing Coenzymes in the Blood in Health and in Various Diseases, in Particular Schizophrenia, Neurocirculatory Asthenia and Tuberculosis. W. Kerppola. *Acta Med. Scandinav.* 153: 33, 1955.

The total and esterified riboflavin content of the blood in 445 patients with various diseases and 60 normal subjects was determined by the method of Fujita and Matsuura. The average normal total riboflavin level was 6.68 1μ per 100 ml, 84 per cent of which (5.51 1μ) was esterified.

In general, in this study from Finland, the blood level in this miscellaneous group of patients was similar to those of the normal subjects. However, in one-fifth of the patients with neurocirculatory asthenia the total riboflavin levels were decreased. Higher values than normal were found in patients with leukemia.

Esterified riboflavin was markedly reduced in schizophrenia, multiple sclerosis, and neurocirculatory asthenia. A lesser reduction of esterified riboflavin was found in peptic ulcer, tuberculosis, rheumatic diseases, bronchial asthma, and chronic colitis. The author concludes that there is no noteworthy riboflavin deficiency in Finland.

It is quite remarkable that no attention was paid to the nutritional status. Except for one sentence that the patients were given ordinary hospital food and no drugs containing vitamin B (*sic*), no data on weight, previous dietary, etc., are given. Furthermore, to conclude that no appreciable riboflavin deficiency exists in a country because the *blood* levels of 500 patients are within "normal" limits seems completely unwarranted.—S. O. WAIFE

Common Electrolyte Abnormalities Encountered in Bowel Surgery. Mechanism of Hypochloremic Alkalosis, Hypokalemic Alkalosis and Hyperchloremic Acidosis. K. E. Roberts, P. Vanamee, H. T. Randall, and J. M. Walker. *Surg. Clin. N. Am.* 35: 1189, 1955.

The most common electrolyte imbalances encountered in gastrointestinal surgery are metabolic alkalosis, and metabolic acidosis resulting from severe diarrhea, small bowel fistulae, or ureterosigmoidostomy.

Metabolic alkalosis results from gastric suction or vomiting (hypochloremic) and from chronic potassium depletion (hypokalemic). The finding of a progressive plasma alkalosis occurring in the face of an acid urine and a low urinary potassium concentration suggests the diagnosis of hypokalemic alkalosis. This situation is refractory to treatment with sodium or ammonium chloride. Combined hypochloremic and hypokalemic alkalosis is common in patients with gastric drainage of several days' duration without adequate therapy.

Hyperchloremic acidosis secondary to ureterosigmoidostomy is seen with increasing frequency as more and more extensive pelvic and rectal operations are being performed. Adequate fluid intake and administration of lactate and bicarbonate are indicated. Hypokalemia may also develop in this group of patients.

and the authors find that potassium as well as bicarbonate supplementation is more satisfactory in patients with an adequate urinary output. There are useful diagrams in this article which illuminate the interesting physiologic discussion.—S. O. WAIFE

The Importance of Nutrition in the Management of Patients with Carcinoma of the Colon and Rectum. W. T. Fitts, Jr., A. F. Zimmerman, and I. S. Ravdin, *Surg. Clin. N. Am.* 35: 1201, 1955.

Carcinoma of the colon and rectum is one of the most common of cancers. The prognosis is fair; about 50 per cent of patients will be well after 5 years, if no metastases are present at the time of operation. The proper nutritional management of these cases is of prime importance.

Anemia is frequently present. The average blood volume deficit in 59 patients was 883 ml. Blood transfusions are recommended irrespective of the cause of the anemia.

Hypoproteinemia is also common. All methods of nutritional correction, including food by mouth, gastric tube feeding, and parenteral alimentation may have to be used. The authors feel that subclinical avitaminosis may often be present and that the added stress of operation and convalescence may precipitate physical signs of vitamin deficiencies. The use of intramuscular preparations is recommended. Vitamin K should be administered until oral nutrition and regrowth of intestinal flora is resumed.—S. O. WAIFE

Nutrition in Gastric Surgery with Particular Reference to Nitrogen and Fat Assimilation. H. Javid. *Surgery* 38: 641, 1955.

Fat and nitrogen metabolism were studied in dogs subjected to various gastric operations. Gastroenterostomy and vagotomy separately had no effect on the percentage of fat and nitrogen output in feces. Vagotomy combined with gastroenterostomy increased fat but not nitrogen excretion. Partial gastrectomy increased the fecal excretion of both substances. The combination of vagotomy and partial gastrectomy increased the fat and nitrogen output to a degree considerably higher than gastrectomy alone. This combination of procedures had the most harmful nutritional effect.—S. O. WAIFE

Content and Distribution of Potassium and Chloride in Adults. D. Ikkos, H. Ljunggren, R. Luft, and B. Sjögren. *Metabolism* 4: 231, 1955.

In 21 adults, 12 males and 9 females, ranging from 22 to 45 years, total body water (TBW) was determined by volume of distribution of deuterium oxide or antipyrine; extracellular water (ECW) by inulin or thiosulfate; exchangeable potassium (K_e) and chloride (Cl_e) by dilution of radioactive isotopes K^{42} and Br^{82}.

The total body water (deuterium) averaged 55.5 per cent of the body weight, or 20.5 liters/sq m surface area. The figures derived from antipyrine are 52.6 and 19.8, respectively. The extracellular water (inulin) was 15.5 per cent of body weight, or 5.8 l/sq m. Using thiosulfate, the figures were 15.1 and 5.6, respectively.

The exchangeable potassium averaged 45 meq per kg, or 1661 meq/sq m; this amounted to 81.2 meq/l of the total body water (deuterium) or 87.9 meq/l using antipyrine. Exchangeable chloride averaged 29.5 meq/kg, or 1087 meq/sq m; this amounted to 53.4 meq/l of total body water (deuterium) or 56.1 meq/l (antipyrine).

These values are subject to a variety of errors, principally that involving inaccuracies in the measurement of the extracellular compartment. However, they may be useful as reference standards when fluid and electrolyte derangements in disease are to be studied.—C. R. SHUMAN

Influence of Dietary Levels of Sodium Chloride and Protein on Growth, Nitrogen Balance and Body Composition of the Growing Rat. *Am. J. Physiol.* 182: 83, 1955.

Young growing rats fed protein *ad libitum* at casein levels of 10, 25, and 40 per cent were observed to have a depression of growth when 15 per cent sodium chloride was added to the diet. The observed decrease in nitrogen retention does not affect weight gain significantly when food intakes are equal, but influences the composition of the gains. Control animals fed *ad libitum* gained more fat, water, nitrogen, ash, sodium and potassium than those on a high salt intake. Pair-fed comparisons were made. Those on a high salt intake gained more than the pair-fed controls. The difference was due to an increased weight of gastrointestinal tract contents. Evisceration made the two groups equal. The inorganic sodium and potassium gains were similar, but animals on high salt intake gained less nitrogen and ash. At the two higher levels of protein, combined with high salt intake, the gain in fat was more depressed than with the 10 per cent casein diet plus salt. Balance studies showed that a high salt diet caused a greater nitrogen digestibility, especially at higher casein intakes. On the other hand, a lesser percentage of nitrogen was retained on a high salt diet than in the case of pair-fed or *ad libitum*-fed control animals.—M. J. OPPENHEIMER

Kidney Glutaminase and Carbonic Anhydrase Activity and Tissue Electrolyte Composition in Potassium-Deficient Dogs. M. Iacobellis, E. Muntwyler, and G. E. Griffin. *Am. J. Physiol.* 183: 395, 1955.

Potassium-deficient diets were fed to dogs for a period of 4 weeks. A second group was fed a diet deficient both in potassium and chloride. If dogs on a low K diet received DCA there was a fall in K, but a rise in Na of skeletal muscle. On this regime plasma Cl and HCO_3 were unchanged. However, if Cl deficiency were added to the previous situation, blood chloride was observed to be depressed while plasma bicarbonate increased. K and Na changes in voluntary

muscle were the same as those when Cl deficiency was not part of the diet. Potassium-deficient rats develop hypochloremic alkalosis. If chloride is adequate dogs are able to maintain an adequate blood chloride level in the face of a K deficiency. Potassium restriction elevates kidney glutaminase activity, but when chloride deprivation is added, then levels of this enzyme are depressed. Activity of kidney glutaminase and urinary ammonia excretion were roughly parallel—kidney carbonic anhydrase activity was related to total titratable acidity in the urine on K-deficient diets. If both K and Cl were restricted, the anhydrase increased but total acidity was observed to be less. DCA reduced kidney carbonic anhydrase activity.—M. J. OPPENHEIMER

Effects of Epinephrine and of Norepinephrine on Carbohydrate Metabolism in the Rat. W. Bloom and J. A. Russell. *Am. J. Physiol.* 183: 356, 1955.

In normal unfasted rats epinephrine increases blood sugar three times more than norepinephrine. Even though the dose of norepinephrine was increased ten times there was little further elevation in hyperglycemia. Norepinephrine did not increase blood lactate even during the period when epinephrine increase in lactate was at its height. Toxic doses of norepinephrine produced a delayed rise in lactate. Diaphragm and gastrocnemius glycogen was depleted by epinephrine, but not by norepinephrine. Neither agent influenced liver glycogen, although both increased the amount of this substance in heart. Demedullation of adrenals did not change the action of the two agents under consideration when the parameters of blood glucose and lactate were examined. After adrenalectomy there was a smaller hyperglycemic response to both agents but changes in lactate after epinephrine were unchanged. During insulin hypoglycemia in fasting rats there was an increase in blood lactate, but glycogen of gastrocnemius was depleted. These last two changes were blocked if the adrenal medulla was destroyed.—M. J. OPPENHEIMER

The Protein-Anabolic Effect of Testosterone Propionate in the Hypothyroid Rat. C. D. Kochakian and J. Dolphin. *Am. J. Physiol.* 180: 317, 1955.

Hypothyroid castrate rats were prepared with thiouracil, and by surgical removal of the thyroid gland combined with castration. In both of these preparations testosterone propionate produced a protein anabolic action. The results were similar to those observed in castrate rats. It was noted that there was a marked decrease in food consumption if the androgen was administered later than 18 days after thyroidectomy. Such was not the case in thiouracil-treated animals.—M. J. OPPENHEIMER

Effect of Injected Choline Citrate in Experimental Atherosclerosis. D. Kritchevsky, A. W. Moyer, W. C. Tesa, J. B. Logan, R. F. J. McCandless, and M. C. Davies. *Am. J. Physiol.* 183: 535, 1955.

Intraperitoneal injections of choline citrate decreased serum cholesterol levels and reduced the atheromata in rabbits which were fed cholesterol. Choline chloride failed to have these actions. Although sodium citrate lowered serum cholesterol it did not appear to influence the atheromatous plaques. All the salts were ineffective by mouth.—M. J. OPPENHEIMER

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Dr. Sure gives a thorough analysis of the challenge of producing sufficient food for the ever-increasing world population. He discusses first the prevalence of hypoproteinosis, then recent methods advocated and tried in improving the world food situation. He considers also the role of various governmental agencies in making available adequate supplies of high quality foods to the malnourished and over-populated nations. Certain endemic anomalies are also discussed.

THE EFFECTS OF SUPPLEMENTARY CALORIES ON NITROGEN STORAGE AT HIGH INTAKES OF PROTEIN IN PATIENTS WITH CHRONIC LIVER DISEASE

Irvin C. Plough, Frank L. Iber, Margaret E. Shipman and Thomas C. Chalmers . . 224

This paper discusses three patients with liver disease in whom large supplements of carbohydrate or fat spared amounts of nitrogen varying from 1.4 to 6.5 mg. per added calorie. In two patients the effects of carbohydrate and fat were equal; in the third patient carbohydrate was twice as effective as fat. The amount of nitrogen spared per added calorie was directly related to the level of protein in the diet. It is suggested that at lower protein intakes an excess of calories leads to the formation of fat, but at higher protein levels leads to the formation of lean tissue.

THE PREVALENCE AND INCIDENCE OF OBESITY IN A CROSS-SECTION OF ELEMENTARY AND SECONDARY SCHOOL CHILDREN

Mary Louise Johnson, Bertha S. Burke and J. Mayer 231

A study of obesity among school children in two communities within the greater Boston area was conducted using school records as a source of information and the Wetzel Grid as the basis for the definition of overweight. The data demonstrated that more than 10 per cent of the children in the area are "overweight." By and large, onset of overweight took place during the winter months, which may be taken as emphasizing the importance of inactivity in the etiology of many cases of obesity.

DIETARY INTAKE AND SOCIAL CHARACTERISTICS

Jean K. Boek 239

In this study of 184 Michigan farmers, the 24-hour recall record of diet was translated into number of calories, milligrams of ascorbic acid, grams of calcium and number of different foods for comparison with each of eight measures of their social milieu. In 12 of the 32 comparisons statistical support was given to the general hypothesis that an association exists between food intake and social environment.

HIGH FLUORIDE CONTENT OF TEETH FROM COMMUNITIES WITH LOW FLUORIDE WATER SUPPLIES

James H. Shaw, Om P. Gupta and Margaret E. Meyer 246

A study of the enamel and dentine of teeth from individuals in Delhi and Bombay revealed a higher fluoride content than would be expected in view of the low fluoride content of the water supplies in these communities. Only part of the difference in fluoride content between the teeth of persons in Boston and those in Delhi and Bombay can be explained by the probable higher water consumption among the Indian citizens. The remainder of the difference would appear to be due to an additional source of fluoride in the diet, with sea salt as a logical possibility, and/or to a higher rate of absorption and utilization of the ingested fluorides.

METHODS FOR PREPARING DIETS LOW IN SODIUM AND HIGH IN PROTEIN

Sherman Beychok, Arthur Kathman, Harry Baron and Israel S. Kleiner 254

Practical methods for removing much of the sodium from various foods by extraction and by dialysis are described. Removal values of sodium from various foods are tabulated. A diet containing as little as 100 to 500 mg. of sodium with a satisfactory protein content may be made up of such foods combined with other low sodium foods. It is palatable and variable, and aids in reducing blood pressure in many instances of hypertension.

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1. Cannon, P. R.: J.A.M.A. *135*:1043, 1947.
2. Flodin, N. W.: American Miller & Processor *81*:30, 1953.

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Margaret McGregor Hard and Nettie C. Esselbaugh 261

Food nutrient intakes from seven-day dietary records are reported for 248 fifteen-and sixteen-year-old native born and reared Washington boys and girls selected from coastal and inland areas of the state. Differences in intakes of calcium, ascorbic acid and other nutrients between the persons in the two areas are discussed, and physical comparisons made in view of these findings.

OBSERVATIONS ON THE URINARY EXCRETION OF THIOCTIC ACID
E. L. Patterson, H. P. Broquist, M. H. von Saltza, A. Albrecht, E. L. R. Stokstad and T. H. Jukes 269

The urine of several animal species, including man, contains an unidentified form of thioctic acid that supports the growth of *Corynebacterium bovis* but not *Streptococcus faecalis*. The thioctic acid activity of the urine showed an increase followed by a return to normal during the first 24 hours after administration of DL-thioctic acid by several routes. The simultaneous administration of thiamine with DL-thioctic acid to man did not alter the thioctic acid activity in the urine. The thioctic acid activity in urine is conjugated probably through one or both of its sulfur atoms. The use of thioctic acid in patients with elevations in blood keto-acids is discussed briefly.

EFFECT OF FOOD INTAKE ON AMINO ACIDS IN HUMAN PLASMA
E. Staten Wynne and Cora L. Cott 275

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THE EFFECT OF ORAL DEXTRAN ON BLOOD GLUCOSE
Robert H. Joelson 279

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Od Kridakara, Rith Boozayaangool, Isra Yuktananda and J. F. Volker 280

Over 2000 Thai school children from 4 to 18 years of age were given dental examinations. Observations were made of prevalence of caries, gingival health and occlusion. The most significant finding was the high caries attack rate in the deciduous teeth of children living in the Bangkok area. It is suggested that this may be due in part to the increased sugar and wheat flour consumption.

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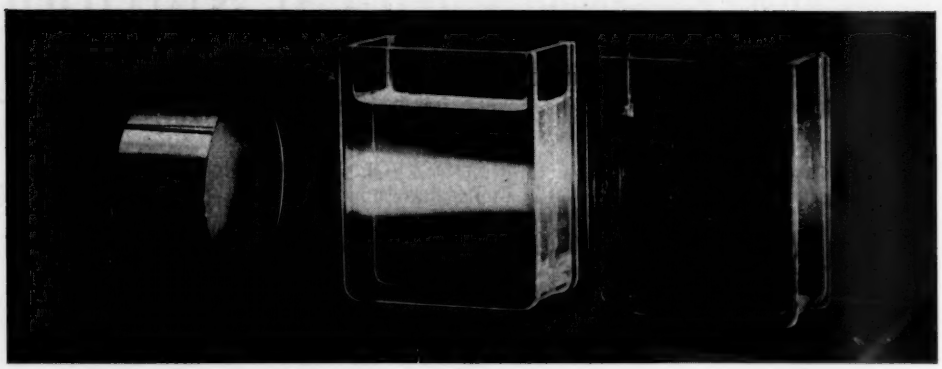

ANOTHER HIGHLIGHT ON LECITHIN – A NATURAL PHOSPHATIDE

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Phosphatides have been found in all vegetable and animal cells. There seems little doubt that they are part of the basic structure of protoplasm and also enter into cell metabolism. The most abundantly found phosphatides are the lecithins, whose surface active properties, when combined with proteins and carbohydrates, play an important role as physiologic emulsifiers of fats and oils.[1]

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Administration: "RG" Lecithin is presented in palatable granules which may be taken plain, in milk, in orange juice or other citrus juice, or sprinkled on cereal.

Literature available on request.

Bibliography: 1. West, E. S., and Todd, W. R.: Textbook of Biochemistry, New York, The Macmillan Company, 1952. p. 184. • 2. Drill, V. A.: Pharmacology in Medicine, New York, McGraw-Hill Book Company, Inc., 1954, p. 64/6. • 3. Ahrens, E. H., Jr., and Kunkel, H. G.: J. Exper. Med. *90*:409 (Nov. 1) 1949.

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